

The University of Chicago

FOUNDED BY JOHN D. ROCKEFELLER

THE EARLY DEVELOPMENT OF
PLANORBIS

A DISSERTATION

Submitted to the Faculties of the Graduate Schools of Arts,
Literature, and Science, in candidacy for the degree of

DOCTOR OF PHILOSOPHY

(Department of Zoölogy)

Oct. 1, 1897

BY

SAMUEL J. HOLMES

BOSTON, U.S.A.

GINN & COMPANY, PUBLISHERS

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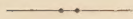
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SAMUEL J. HOLMES.

CONTENTS.

PART I. DESCRIPTIVE PORTION.		PAGE
Methods		370
Nomenclature		373
The Eggs and Egg Masses		373
The First Cleavage		375
The Second Cleavage		376
The Third Cleavage		378
From the Eight to the Twenty-four Cell Stage		379
Cleavage Cavity and Vacuoles		385
From the Twenty-four to the Forty-nine Cell Stage		386
The Division of the Second Quartette and the Formation of the Cross		386
The Trochoblasts		387
The Division of the Third Quartette		390
The Formation of the Primary Mesoblast		391
The Fourth Quartette		393
The History of the Cross		393
The Second Quartette		403
The Mesoblastic Bands		407
The Third Quartette and the Secondary Mesoblast		408
General Considerations on the Secondary Mesoblast		411
The Entomeres		413
The Rudiments of the Cerebral Ganglia and Eyes		415
The Apical Plate		416
The Cell Lineage of the Head Vesicle		417
The Cell Lineage of the Prototroch		418
The Shell Gland and the Foot		421
The Larval Kidney		422
Gastrulation; Fate of the Blastopore		425
PART II. GENERAL CONSIDERATIONS.		
Reversal of Cleavage and Reversed Asymmetry		426
The Relation between Reversed Cleavage and the Direction of the First Cleavage Plane		429
General Considerations on Spiral Cleavage		430
Reversal of Cleavage and Cell Homologies		440
Bibliography		445
Explanation of the Figures		450

THE investigation, the results of which are recorded in the present paper, was carried on under the supervision of Prof. C. O. Whitman, at the University of Chicago and at the Marine Biological Laboratory at Woods Holl, Mass. It is a source of gratification to acknowledge the generous treatment I have received in both these places at Professor Whitman's hands. I wish also to express my appreciation of the many suggestions I have received from Dr. E. G. Conklin, both in person and through his admirable paper on the "Embryology of *Crepidula*."

The species studied is *Planorbis trivolvis* Say. This species occurs in great abundance in the ponds of South Park, Chicago, and it was also found in a pond near Falmouth, a few miles from Woods Holl.

PART I. DESCRIPTIVE PORTION.

Methods.

The eggs of *Planorbis*, when brought into contact with water, swell quite rapidly, so it is best to tease them out of the capsules directly into fixing fluid. The eggs may, however, be teased first into normal salt solution, to which it is better to add a small quantity of the fixative, and then treated with the fixing fluid alone. Some fixing fluids coagulate the albuminous substance around the egg so quickly, after or during its escape from the capsule, that it usually becomes surrounded with more or less coagulated albumen, from which it is difficult to free it. By teasing directly into normal salt solution, mixed with only a small quantity of the fixative, the eggs may be obtained free from any foreign material. Normal salt alone often causes more or less swelling.

Formalin does not appear to coagulate the albuminous substances in the capsules. The egg masses may be kept in a 5% solution of this substance for several days, the jelly remaining perfectly fluid and transparent. Unfortunately, formalin does not otherwise prove a satisfactory fixing agent. Kleinenberg's stronger picro-sulphuric gave good results, especially when followed by the method of staining with acidified Delafield's

haematoxylin, used by Conklin. Lithium carmine proved a good nuclear stain when the eggs were first overstained, and the color extracted by a long treatment with acid alcohol. Haematoxylin has the disadvantage, for the later stages of cleavage, of staining very intensely the small globules of albuminous material, which become very numerous and render the nuclei difficult to observe.

By far the most useful reagent that was employed was silver nitrate. In fact, were it not for the beautiful and clear preparations obtained by using this stain, I should probably have been unable to follow the cell lineage of *Planorbis* to the stages here described. The eggs are teased from the capsules directly into a .75% solution of silver nitrate, and exposed in a watch glass to the sunlight, the brighter the better. The eggs may be examined from time to time with the microscope, and when the cell boundaries stand out clearly, the nitrate is removed and the eggs washed quickly in water. The water being mostly removed, a few drops of a $\frac{1}{8}$ % solution of hyposulphite of soda is added and allowed to act only three or four seconds. In fact, I begin to remove the hyposulphite as soon as it is poured on the eggs. The object of this treatment is to prevent after-blackening of the eggs by dissolving out the unreduced silver. Otherwise the eggs are liable to become in time so dark that they are useless for study. A too prolonged treatment with the hyposulphite, on the other hand, will destroy the silver stain entirely. I have found it unsafe to wash the hyposulphite out with water. The eggs often suddenly swell to twice their normal volume and are thereby spoiled. Instead of water, a saturated solution of picric acid may be used. This acts at the same time as a fixative and does not injure the stain. After a few minutes' treatment with this reagent, the eggs may be passed through the grades of alcohol, cleared in xylol, and mounted in balsam.

I have used to support the cover glass strips of paper of the proper thickness gummed to the slide. By moving the cover, the eggs may be rolled so that they can be studied from all sides. When the balsam becomes hard, it can readily be softened by applying a drop or two of xylol to the edge of the cover glass.

Eggs treated by the foregoing method will keep indefinitely, neither fading nor becoming opaque. When the egg is mounted, the stain is usually safe. In successful preparations the cells of the egg are not strongly darkened, but the cell boundaries stand out in a remarkably sharp and clear manner. Some cells, however, stain much darker than others. The trochoblasts and the cells of the head vesicle remain almost perfectly transparent, while the cells of the cross are colored brown. Owing to the transparency of the trochoblasts the cross appears with a wonderful distinctness, enabling one to orient the egg at a glance. Nuclei are not stained, but they can usually be seen, and the spindles of dividing cells are often visible. The eggs may, however, be stained so that the nuclei show fairly well, but I have generally dispensed with nuclear staining when using this method. The treatment often injures the impregnation and renders the egg more opaque, so that it is more often a nuisance than a benefit. Individual differences in the impregnation of different eggs are quite decided. Even among eggs from the same capsule subjected to exactly the same treatment, some will be strongly stained, while the staining in others is faint. If eggs are left too long in the nitrate, they not only become too dark, but also become brittle, and the cells break or become separated when the eggs are rolled. Cell boundaries take the stain at all periods in the development of the egg, even in the very first cleavage stages. The method is of special value, however, in following the cell lineage of organs in the later periods of cleavage. I have obtained wonderfully clear preparations of gastrulae, which show the boundaries of each cell, when there are several hundred cells in the egg, with diagrammatic distinctness. The cells of the proto-troch in the gastrula stage form a conspicuous transparent band, which appears in marked contrast to the adjacent cells—a circumstance which proved very helpful in tracing out the cell lineage of this organ.

Nomenclature.

In the matter of nomenclature I have followed the system used by Conklin in his paper on the "Embryology of Crepidula," as this method enables one readily to follow the type of cleavage found in annelids and mollusks. Besides, the comparisons constantly to be made throughout this paper, with the work of Dr. Conklin, render it highly desirable, aside from other considerations, that the same system of nomenclature be employed. The word "quartette" is used to designate the products of a generation of cells given off from the four cells at the vegetal pole of the egg. The term "quartette" has been used, however, in a different sense by several writers, who employ it to designate any four cells of radially symmetrical origin. Thus, according to the latter usage, the four outer cells arising from the division of the first generation of ectomeres would constitute one quartette, and the four apical cells another, while, according to the usage here employed, all of the eight cells resulting from this cleavage would still belong to the same quartette. As a substitute for the word "quartette" in the latter sense, the word "tier" has been employed; thus the products of cleavage of the first generation of ectomeres would be called the upper and lower tiers of the first quartette.

The different quartettes in Conklin's scheme are designated by coefficients, and the genealogy of the cells of a quartette is indicated by exponents. The upper cell, or the right one when the cleavage is equatorial, is indicated by the smaller exponent; $2a^1$, for instance, indicates the upper cell in the a quadrant of the second quartette, $2a^2$ the lower. The upper product of the cleavage of $2a^1$ would be $2a^{1.1}$, while $2a^{1.2}$ would represent the lower cell.

The Eggs and Egg Masses.

The egg masses of Planorbis are flattened and rather firm, and are usually found adhering to stones or aquatic plants. The eggs proper are found in relatively large capsules, which are imbedded in a jelly-like mass, outside of which is a somewhat tough enclosing membrane. The amount of jelly between the

capsules is quite small; in fact, the sides of the capsules are generally in contact, leaving only the interstices to be filled by this material. The amount of albumen in the capsules, in comparison with the size of the egg, is, on the other hand, very large. The diameter of the egg measures about .13 mm., while the diameter of the capsule is .6 mm. The egg mass is of a yellowish color in the species studied, and sufficiently transparent to enable one to successfully study the living embryo *in situ*. There is less jelly than in the egg masses of *Physa* and *Lymnaea*, and the membrane surrounding the capsule is less tough. The eggs may be readily teased out of the capsules, whereas in *Physa* and *Lymnaea* the capsules come out of the jelly entire, and slip around like rubber balls when the attempt is made to tease them apart by needles.

The time during which *P. trivolvis* lays its eggs extends from early spring until the fall. The eggs are deposited in the greatest abundance in the spring. Where the snails are abundant, stones may frequently be found almost entirely covered by the egg masses. Often the snails themselves have several clusters of eggs attached to different parts of the shell, and sometimes the egg masses may be found adhering to the bodies of aquatic insects. When kept in an aquarium, the snails, in the early part of their laying season, readily deposit their eggs on the glass; but later in the year, when the eggs are produced in less abundance, the animals become apparently more particular as to where they lay, and seldom deposit their eggs unless upon stones or aquatic plants, upon which they find minute algae, which serve for food.

Usually a capsule contains but one egg, but sometimes it may have two, and rarely three or more. When there are more than one egg in a capsule, often only one of them develops normally, yet two embryos in a late stage of development may sometimes be found in the same capsule. It is common to find some of the eggs in a cluster developing abnormally, and the proportion of such eggs is increased when the water becomes contaminated. If eggs are obtained from snails kept in an aquarium, care has therefore to be taken that the water is kept fresh and pure. With proper feeding and attention,

Planorbis can be made to lay eggs even in the winter months. Some of the snails I kept in glass dishes deposited eggs in the latter part of January, and the cleavage of these eggs was perfectly normal.

The unsegmented eggs of Planorbis are almost spherical in form and of a bright yellow color. The yolk, which gives the egg its yellow color, is somewhat more dense at the vegetal pole, although the difference is not strongly marked. The fresh egg, when seen through the microscope by transmitted light, shows a somewhat darker lower pole—the future entoderm—shading gradually into a lighter upper hemisphere—the future ectoderm. The opaque matter of the egg consists of two elements, small granules and larger globules of deutoplasm. The spheres of deutoplasm are found scattered through all portions of the egg with the exception of a very small, clear, protoplasmic area at the animal pole. The polar bodies are small and clear. The first one is the larger and of almost spherical form, and is carried on the top of the second polar globule. The polar bodies remain in connection with the egg until quite a late period of cleavage, when they drop off and disappear. The nucleus is situated in the small protoplasmic area at the animal pole.

The First Cleavage.

At the beginning of the first cleavage the clear protoplasmic area at the upper pole of the egg increases in size and elongates as the chromosomes are separated. The asters form dense radiating masses of fibers which are clearly visible in the living egg. Their general appearance is very similar to those of Physa, which are figured by Kostanecki ('96). The cleavage furrow appears first at the animal pole and gradually extends downward on either side, finally surrounding the egg. The constriction is deeper at the animal pole, as is the rule with yolk-laden eggs in which the spindle usually lies above the center. After the separation of the parts of the egg is complete, the daughter-cells become nearly spherical in form and come in contact at only a small portion of their surface.

The two cells are equal in size, as is the rule in gastropod eggs in which there is not a very large amount of yolk. The nuclei are large and vesicular, containing, in proportion to their size, only a small amount of chromatin. The yolk spheres again encroach upon the protoplasmic area around the nuclei, from which they had been extruded during mitosis, and the cells gradually flatten against each other until they resemble a single undivided sphere. The behavior of the yolk in relation to the processes of cell division seems to indicate that the regions around the poles of the spindle are the seat of a tension which squeezes out the deutoplasm spheres as the contraction of a sponge would squeeze out the water contained in its meshes. With the disappearance of the astral radiations and the decrease of tension in those regions, the yolk spheres become free to distribute themselves more uniformly through the egg. It seems probable that the rounding off of the cells after division is due to the persistence for a time of the same central tension which excluded the deutoplasm spheres from the region of nuclear division, and that the subsequent flattening of the cells after the resting period has begun is due to a relaxation of the tension which at the same time permits the more even distribution of the yolk, and allows those agencies tending to draw the cells together to become dominant. This flattening of the two blastomeres upon each other occurs very soon after their complete separation, and it continues until each blastomere becomes almost a hemisphere. A lenticular cleavage cavity, if we may call it such, makes its appearance at this stage, reaching its maximum development just before the next cleavage.

The Second Cleavage.

The two cells usually begin to divide at the same time. The division of one cell sometimes occurs a short time before that of its fellow, but the cleavage is never completed before the process of division in the other cell is well under way. Both spindles are at first parallel to the plane where the cells join. When the elongation of the cells occurs, the spindles

turn slightly towards the right, and the cells themselves, as they lengthen, undergo a twisting around in the same direction. The first cleavage plane, when viewed from the animal pole, is bent first to the left and then to the right. The reason of this is that the cleavage is not perfectly horizontal, but two of the cells lie a little higher than the others. When the division is completed, there result four cells nearly equal in size, two of which, *B* and *D*, come in contact below, in the ventral cross furrow, while the other two, *A* and *C*, meet in a cross furrow at the upper pole, which is nearly at right angles to the lower one. The ventral cross furrow makes a negative angle of about 45° with the first cleavage plane, while the upper cross furrow makes with this plane an equal positive angle. The bending of the first cleavage furrow at the lower pole of the egg is the reverse of what occurs in *Crepidula* and other mollusks with dextrotropic cleavage. It is a rule, holding good for all known cases, that in dextrotropic cleavage the ventral cross furrow, when viewed from the animal pole, bends to the right, while in forms with reversed cleavage it bends to the left. The cross furrow at the animal pole of the egg, however, does not show such a constant relation to the first cleavage plane. In many cases it may be absent entirely, the four blastomeres meeting above in a point. In some cases it is parallel to the ventral cross furrow (*Crepidula*), in others it is nearly at right angles to it (*Nereis*, *Planorbis*, *Physa*, *Lymnaea*). These variations obviously depend upon the fact that in some cases the two cells *B* and *D* meet above as well as below,—in which case the two polar furrows would be parallel,—while in other eggs the alternate cells *A* and *C* meet at the animal pole and form a cross furrow at right angles to the lower one. It is not surprising, therefore, that the polar furrows should sometimes present different relations to each other in eggs of the same species.

The four cells round off after division like those produced by the previous cleavage. They become almost spherical, but they subsequently draw together and assume very nearly the form of a single undivided sphere. A cleavage cavity occurs between the two pairs of cells *AB* and *CD*; that is, between

those cells which were not separated by the last cleavage. In addition, a central cleavage cavity occurs somewhat later which reaches a considerable size and assumes a quadrilateral form. All of these cavities disappear during the next cleavage.

The Third Cleavage.

The third cleavage in *Planorbis* is laetotropic. As in some other forms, the amount of rotation is more pronounced during the later stages of cell division, and the cells of the upper quartette finally lie in the angles between the larger lower cells. After the shifting has taken place the cells flatten, and there results once more an almost spherical mass of cells. A central cleavage cavity again makes its appearance, and again disappears during the next cleavage. The four upper cells resulting from this division, or the first quartette of ectomeres, are formed of clear granular protoplasm, which gives them an appearance quite distinct from the lower cells which contain the yolk. While much smaller than the lower cells, or macromeres, they are considerably larger than the corresponding cells in the eggs of most gasteropods. The cells 1*a* and 1*c* meet in a polar furrow, which is inclined at a considerable angle to the polar furrow at the vegetal pole.

It seems probable, as suggested by Lillie, that Rabl has made some errors in the orientation of the eggs figured in his first plate, which tend to produce confusion regarding the relation of the two polar furrows. In the first place, Rabl's figures indicate that, in the four-cell stage, the upper and lower cross furrows are parallel, which is the reverse of what occurs in the species here described. In case *B* and *D* meet above, as well as below, we might expect that their derivatives, 1*b* and 1*d*, would also meet in a cross furrow, whose angle with the lower furrow would approximately measure the amount of rotation of the micromeres. Kofoed's Fig. *D* ('95, p. 53) would represent their relation under such a supposition. Rabl's Fig. 11*A* indicates a further rotation of the upper polar furrow until it lies at right angles to its original position. Fig. 12*A*, on the other hand, shows this furrow at right angles to its position in 11*A*.

There is doubtless an error in the orientation of this figure, if not in the figures of the cross furrow in some of the preceding stages.

In *P. trivolvis* the upper polar furrow would lie at right angles to the lower one, were it not that the shifting of the micromeres from right to left lessens this angle to one of about 45° . The eight-cell stage in *P. trivolvis* differs from that figured in Kofoed's Fig. *D* in that the upper polar furrow lies between *1a* and *1c* (or the cells which Kofoed has called $b^{4.2}$ and $d^{4.2}$) instead of *1b* and *1d* ($a^{4.2}$, $b^{4.2}$, Kofoed), and is placed at right angles to the one there figured. The upper polar furrow makes a positive instead of a negative angle of 45° with the lower polar furrow taken as an axis.

From the Eight to the Twenty-four Cell Stage.

The four macromeres are the next cells to divide. This cleavage is dextrotropic, *i.e.*, in the reverse direction to the previous one. The second quartette of ectomeres thus formed is, like the first, composed of rather large clear cells, which, however, are markedly smaller than the macromeres. The cells flatten out after division, as after the preceding cleavages, so that the furrows between the cells almost disappear, and the cell outlines are marked only by narrow clear lines.

From the twelve-cell stage the egg passes quickly to that of twenty-four cells. The first quartette of ectomeres are the first cells to divide, the division occurring in a right-handed spiral. Very soon spindles occur almost simultaneously in the cells of the second quartette and in the macromeres. The cleavage of the cells of the second quartette is laeotropic, and the resulting cells are nearly equal in size. The division of the macromeres, which is likewise laeotropic, gives rise to the third generation of ectomeres. The cells of the third generation are large, and are marked off sharply from the macromeres, even before the division is completed, by their clear protoplasm. From their superficial outline they appear equal in size to the macromeres, or even larger. Their actual bulk, however, is less, as may be seen in optical sections, for they do not extend so far into the interior of the egg.

The twenty-four-cell stage, which is reached by these divisions, marks a resting stage of considerable length in the development of the egg. A cleavage cavity is formed at this period, which may attain quite a large size. In the arrangement of the cells the egg presents a perfect radial symmetry. The macromeres, or entomeres, as it is better now to call them, are somewhat larger than the cells above them, and their yellowish color and greater opacity, due to the yolk they contain, render them easily recognizable. Lying in the angles between the four entomeres are the cells of the third quartette. These cells are elongated in a meridional direction, the direction of their next cleavage. Alternating with these cells, and hence lying opposite the entomeres, are the lower cells of the second quartette, $2a^2$, $2b^2$, $2c^2$, $2d^2$. The four entomeres are, therefore, surrounded by a circle of eight cells, of the second and third quartettes. The four upper cells of the second quartette, when viewed from the upper pole, lie a little to the left of the lower ones, and do not come into contact with the cells of the entoderm. At the upper pole of the egg there are eight cells, the products of the cleavage of the first quartette of ectomeres; the four lower cells, the trochoblasts, alternate with the cells of the second quartette, and lie opposite those of the third with which they are in contact. The relation between these cells corresponds essentially to the relation between the groups of cells arising from them, even in a late stage of development. The descendants of the four upper cells go entirely into the formation of the cross, which will be described later; below these cells lie those of the second quartette, and below these again the entomeres. Alternating with these groups, there occur, in vertical arrangement, the trochoblasts, the third quartette, and the angles between the entomeres. This arrangement of the various quartettes, which is typical for molluscan spiral cleavage, is a great aid in following their further history.

There are a few points in which the cleavage of *P. trivolvis* differs from Rabl's account of the cleavage of the form studied by him, which it may be well to point out. Rabl's different account of the cross furrows has already been discussed. The passage from the twelve to the twenty-four cell stage takes

place, according to Rabl, by the simultaneous division of all of the cells of the egg. In *P. trivolvis* the first quartette divides before the others, forming a sixteen-cell stage. Finally, although Rabl says nothing concerning the direction of the cleavage of the cells of the second quartette, his Figs. 12*A* and 12*B* indicate that the division was dextrotropic. In *P. trivolvis* this cleavage is plainly laeotropic, and the cells lie much more nearly in a vertical plane than they are represented in Rabl's figure. There can be no doubt that the first quartette in the forms studied by Rabl is given off in a laeotropic direction. He expressly states this, and it is also indicated by his figures. The second quartette, according to the law of alternation of spirals, should be given off in a dextrotropic direction. If, as Rabl's figures indicate, the second quartette divides dextrotropically, there would result two dextrotropic divisions in immediate succession. It seems more probable that Rabl's figures are misleading on this point than that there should be an exception to the law of alternation of spirals at this early period of cleavage. It is not unnatural that, not having in mind any significance attached to this point, an observer should be in error regarding it.

The cleavage of the eggs of *Physa* and *Lymnaea* as far as the twenty-four-cell stage is essentially the same in almost every point as that of *Planorbis*, and there is a remarkable agreement between the cleavage of *Planorbis* and *Limax*, as described by Kofoed and Meisenheimer, as far as the cell lineage in the latter forms was carried. The cleavage of the eggs of the pulmonates, so far as they have been studied, seems, in fact, to be characterized by several points of marked similarity. The amount of yolk in the eggs is not great; the ectomeres are large; there is a recurrent cleavage cavity; and vacuoles are often formed between the blastomeres. In the twenty-four-cell stage in the above forms the cells have essentially the same relative size and arrangement, and the entomeres are scarcely larger than the other cells of the egg. The eggs of pulmonates complete their development in capsules which contain a very large amount of fluid albuminous substance, which serves to nourish the embryo. As the amount of food in the form of albumen is large, it is

natural that the amount of food in the form of yolk should be small, and the more nearly equal size of the blastomeres in the early cleavage stages of pulmonates, in comparison with the eggs of most marine forms, is probably due to the relatively small amount of yolk in the egg. It is the rule among gastropods that the greater the amount of yolk in the egg the smaller are the ectomeres in relation to the entomeres. A comparison of such yolk-laden eggs as those of *Purpura* and *Nassa* with the eggs of *Crepidula* and *Umbrella*, or these again with the eggs of *Paludina* or the pulmonates, will illustrate this principle very forcibly. It has been pointed out by Kofoed, as a rule holding for a great variety of forms, that "the greater the amount of yolk, the greater seems to be the tendency of the cells of a yolk-laden quartette to divide before those of the smaller quartette." For instance, in the eggs of *Limax*, which have little yolk, both macromeres and ectomeres at the eight-cell stage divide almost simultaneously, and the egg passes at once to the sixteen-cell stage. In the eggs of *Planorbis*, *Physa*, and *Lymnaea*, which contain somewhat more yolk, the macromeres divide before the ectomeres, giving rise to a stage with twelve cells. In the eggs of *Umbrella* and *Urosalpinx*, which contain a still larger amount of yolk, even the third quartette is given off before the first has divided. These facts can scarcely be said to show, however, that the presence of yolk in cells actually accelerates their division, as Kofoed seems to imply. It is a general rule that the larger a cell is, the sooner it tends to divide. There are numerous exceptions to this rule, some of which will be pointed out later, but it expresses a more or less dominant tendency in the cleavage of the egg. A large amount of yolk in the egg, moreover, would determine the micromeres to be of small size, and the small size of these cells would tend to delay their cleavage. The yolk may, and probably does, delay the cleavage of the cells containing it, but the small size of the ectomeres in yolk-laden eggs delays their cleavage even more. It is probably for this reason that we find a delayed cleavage of the ectomeres in yolk-laden eggs. And this conclusion is in harmony with the fact that the cleavage in yolk-laden eggs is usually slow.

As, perhaps, in all other mollusks, except the cephalopods, and in the annelid worms, all of the ectoderm is contained in the first three quartettes of micromeres. The three macromeres, *A*, *B*, and *C*, are entirely entodermic, while the posterior one, *D*, contains both entoderm and mesoderm. The cases in which more than three quartettes of ectomeres are said to be formed I think we must regard, with Conklin, as open to serious question. In the case of *Fulgur*, in which a large number of quartettes of ectomeres was said to be formed, Conklin has shown that there is, in reality, only the usual number, three. In *Nassa*, Bobretzky held that the macromeres budded off a large number of ectomeres; but Conklin finds that in the closely related genus, *Ilyanassa*, the usual number of quartettes is produced. Salensky has asserted that more than three quartettes of ectomeres are formed in *Vermetus*, and Erlanger has made the same statement regarding *Bythinia*. But in *Serpulorbis squamata*, from the coast of California, — a form very closely allied to *Vermetus*, in which genus it was, in fact, originally placed, — I have found that the whole ectoderm arises from but three quartettes.

It is not so much the fact that the cases reported of the formation of more than three quartettes of ectomeres are exceptions to a general rule that makes them so improbable — it is the very definite and similar fate of these quartettes in all the forms in which their history has been traced. Speaking of this rule that the ectoderm in annelids and mollusks arises from three quartettes, Dr. Conklin says, "The cause of this remarkable phenomenon is to be found in the fact, I believe, that each of these quartettes of ectomeres is the protoblast of definite regions of the embryo." Each quartette forms essentially the same parts of the embryo in every mollusk that has been studied in this regard. An additional quartette would necessitate a considerable modification of the fates of the preceding quartettes. Were the fates of the different cell generations to a large extent indeterminate, a variation in their number would not seem so great an improbability. Since, however, each quartette has essentially the same destiny, not only in widely separated groups of the Mollusca but also in annelids, we have very strong reasons,

I believe, for holding the accounts of the formation of four or more generations of ectomeres to be erroneous.

The last author who records more than three generations of ectomeres is Fujita, who studied the cleavage of the pulmonate Siphonaria. After describing the formation of the four-cell stage, he says: "During the next following stages four successive generations of micromeres are budded off from each of the above-mentioned segments, now to be called macromeres. Hereupon the macromere *D* is entitled to the name of entomesoderm, and the remaining three macromeres may be called entodermic macromeres. Synchronously with the formation of the fourth generation of micromeres, each member of the third generation divides, thus giving rise to a fifth generation. At this stage there are twenty micromeres and four macromeres, the relations of which may be seen in Fig. 2. Next comes in order the formation of a sixth generation of micromeres again from the third, followed by that of a seventh from the fifth." It seems to me quite certain that Fujita overlooked the division of the first generation of micromeres and concluded that the four outer cells resulting from this cleavage arose from the macromeres. This is a very natural and easy error to make, as I can testify from experience, having been deceived, for a time, on just this point, when working on another form. Fujita describes no division of the first quartette until after the stage in which the egg contains thirty-four cells, when the cells of the second quartette have divided twice, and the posterior cells of the third have divided. Moreover, Fujita's figure of the twenty-four-cell stage shows that it corresponds exactly, so far as the relations of the cells are concerned, with the same stage in *Planorbis*, *Physa*, and *Crepidula*. If we assume that the cells marked 2 in Fujita's Fig. 3 are the trochoblasts, the genealogy of all the cells would exactly correspond to that in the above forms, and the following divisions up to the forty-third-cell stage, which Fujita describes, would correspond point for point with those of *Planorbis*. The cells 2 are smaller than the apical cells, and their position indicates that they arose by a laeotropic division, as would be expected according to the principle of alternation of spirals. All of these

facts, taken in connection with the antecedent improbability of Fujita's conclusion, make it appear very probable that the egg of Siphonaria contains but the usual number of quartettes of ectomeres.

Cleavage Cavity and Vacuoles.

The existence of a recurrent cleavage cavity seems to characterize, in an especial manner, the cleavage of the pulmonate gasteropods. It has been observed by several workers on these forms (Warneck, Fol, Rabl, Brooks, Schmidt, Meisenheimer), and has been described so fully in the case of *Limax* by Kofoid, that it will only be briefly considered here. In *Planorbis* the cleavage cavity does not attain nearly such extensive development as in *Limax*. Whether this relation obtains between the aquatic and terrestrial pulmonates in general is uncertain, though in the aquatic forms, *Physa*, *Lymnaea*, and *Planorbis*, the cleavage cavity is not so large as in the terrestrial genera, *Limax* and *Succinea*. In *Planorbis*, as in all the above forms, the cleavage cavity first appears in the two-cell stage. This disappears at the next division; and, in the four-cell stage, two cavities appear between the two pairs of cells *AB* and *CD*, and, in addition, there develops a central cleavage cavity which assumes a quadrate form and finally merges with the other two. A similar occurrence repeats itself at the eight-cell stage. With each cleavage the cavity disappears, then forms again, and gradually increases in size until the rounding off of the cells during the next cleavage causes a break in the wall and allows the fluid contents to escape. From the twenty-four-cell stage on, the cleavage cavity appears to be permanent.

Small intercellular vacuoles occur in a late period of cleavage, as in *Limax*, and they are found most abundantly in the ectodermic portions of the egg. All of these cavities, as pointed out by Kofoid, seem to be the result of excretory activity. (For a discussion of the function and occurrence of the cleavage cavity in different forms, see Kofoid ('95), p. 81.)

From the Twenty-four to the Forty-nine Cell Stage.

The Division of the Second Quartette and the Formation of the Cross. — The transition from the twenty-four to the forty-nine cell stage occurs very quickly. The upper tier of the second quartette divides in a dextrotropic direction. The upper cell, as in *Crepidula*, *Umbrella*, and *Unio*, is the smaller, and Meisenheimer's Fig. 31 shows the same is true also in *Limax*. These upper cells, as in the above forms, form the tips of the arms of the cross presently to be described. Blochmann was doubtless wrong in his derivation of these tip cells in *Neritina*, and I think Conklin's correction of this mistake is to be followed, rather than that given by Kofoid, as it brings the cleavage of *Neritina* into complete harmony with that of other mollusks (see Conklin ('97), p. 64). The tip cells of the lateral arms of the cross in *Neritina* were found by Blochmann to have a peculiar granular appearance, and were held by him to give rise to the velum. It is probable, however, that only a portion of the velum is formed from these cells, as in both *Crepidula* and *Planorbis*, and also in *Ischnochiton*, certain cells of the first quartette, and other cells from the second also, go into the formation of this organ. The granular character of these tip cells in *Neritina* has not, I believe, been seen in any other form.

The division of the upper tier of the second quartette is soon followed by that of the lower. This cleavage is also dextrotropic, but the smaller cell is now the lower one. The upper cell, resulting from this division, lies at the side of the lower cell, arising from the previous division. There are now four groups of four cells each, or sixteen cells, in the second quartette. In each group there is a pair of large cells situated side by side, and a smaller cell above and one below. The lower cells lie opposite the entomeres.

The apical cells of the first quartette now divide in a laeotropic direction. When this division is completed, the arrangement of certain cells of the upper pole becomes such as to give the appearance of a cross. The outer cells of the first quartette, $1a^{1,2}$, $1b^{1,2}$, etc., form the bases of the arms of the cross,

the inner ones forming the center, and the cells of the second quartette forming the tips. At its first appearance the cross, therefore, contains twelve cells, eight belonging to the first, and four to the second quartette. From this stage until the period of gastrulation, the cross is a very conspicuous feature of the egg. Its further history will be traced in a later section.

The Trochoblasts.—The cleavage of the apical cells of the egg is soon followed by a division of the four outer cells of the first quartette, the trochoblasts, $1a^2$, $1b^2$, etc. This division is nearly radial, the upper cell lying in the angles between the arms of the cross directly above the lower one. The two cells are about equal in size and they soon begin to enlarge and become clear. One peculiarity of this cleavage is that it occurs at a much earlier stage than in *Umbrella* and *Crepidula*. In the first genus the trochoblasts do not divide until the egg contains more than seventy cells; in the second the anterior trochoblasts do not divide until the number of cells in the egg is over one hundred, while the posterior trochoblasts do not divide until later, if at all. In *Unio*, however, they divide at about the fifty-cell stage, and at a still earlier stage in *Chiton* (Metcalf) and *Ischnochiton* (Heath). If we compare the egg of *Planorbis* with that of *Limax* in this respect, we find a close agreement. The time at which this division takes place in *Limax* is, according to Kofoid, quite variable, but it occurs, speaking roughly, at about the forty-cell stage. The cells in *Limax* are comparatively large, as in *Planorbis*, but, strangely enough, they divide in an entirely different direction. "The axis of the spindle," says Kofoid, "lies parallel to the plane of the equator. There is every indication that the division is nearly meridional (horizontal)." Meisenheimer's Fig. 31 shows horizontal spindles in all four trochoblasts in the egg of *Limax maximus*. The cells resulting from this division lie nearly side by side, instead of the one above the other, as in *Planorbis*, there being only a slight dextrotropic tendency in the cleavage (see Kofoid, '95, p. 59, Fig. 41). In Conklin's Fig. 50 the cells of the two anterior pairs of trochoblasts lie in nearly the same horizontal plane, and their symmetrical position in relation to the anterior arm of the cross indicates that they were produced

by a bilateral division. At a late period the anterior trochoblasts in *Planorbis* become so shifted as to lie in very nearly the same position as in *Crepidula*. It is probable that there is no other group of cells which presents, in different mollusks, such a remarkable degree of variation, both in the time and in the direction of their cleavage. Yet they have essentially the same fate not only in mollusks, but also in annelids. There is, I believe, no reasonable escape from the conclusion expressed by Conklin, that the trochoblasts in annelids and mollusks are truly homologous. Having the same origin, position, and fate in both these groups, the evidence of their homology is as complete as ontogeny can furnish.

In most gasteropods the trochoblasts are of small size. This is especially the case in *Neritina*, *Umbrella*, and *Crepidula*, and, according to Conklin, they are small also in *Urosalpinx* and *Fulgur*. In the pulmonates, however, their size is larger. In the preceding cases the cells of the first quartette are very small in relation to the macromeres and they divide unequally, the outer cells being much smaller than the apical ones. In *Limax agrestis* (Kofoid) and in *Planorbis* the cells of the first quartette are not only much larger than in the above forms, but they divide into almost equal parts. In the twenty-four-cell stage of both forms the trochoblasts have about the same size as the other cells of the egg. Their bulk is, therefore, vastly greater than that of the corresponding cells in *Umbrella* or *Crepidula*. Has not the relatively large size of the trochoblasts in *Limax* and *Planorbis* some causal connection with their precocious divisions? Is not their size dependent largely on the size of the cells of the first quartette, and this, again, upon the relatively small amount of yolk in the egg? It seems probable that the amount of yolk in the egg may indirectly influence the size of the trochoblasts and the time at which they divide. It is not contended that this is the only factor in the case. It does not explain, for instance, why the cells of the first quartette divide almost equally in some cases and very unequally in others. But when we compare the eggs of *Unio*, *Planorbis*, and *Limax* with those of *Umbrella* and *Crepidula*, it is difficult to resist the impression that yolk is, in great measure, respon-

sible for the great difference in the period at which the division of the trochoblasts occurs.

It might be urged that if the large size of the trochoblasts were the cause of their early division, in forms with a relatively small amount of yolk, they ought to continue to divide, since they grow more rapidly than perhaps any other cells of the egg. In *Crepidula* these cells are at first "much the smallest cells of the entire egg"; finally they become, with the exception of the yolk cells, the largest cells of the egg; and their greater relative size is due not only to the fact that the other cells are getting smaller as division proceeds, but their absolute bulk is greatly increased. Yet neither in *Crepidula* nor in any other gasteropod has more than one cleavage of these cells been observed, while in *Ischnochiton*, according to Heath ('99), and in *Amphitrite*, *Lepidonotus*, and *Clymenella* among annelids, according to Mead, they stop dividing entirely after the second cleavage. The cessation of the cleavage of these cells is doubtless connected, as Conklin maintains, with their prospective destiny. They soon acquire cilia and become a part of a specialized organ. This necessitates a special modification of their structure, which, setting in at an early period, checks the tendency to division. It is well known that when the differentiation of a cell is carried very far it generally ceases to divide, or if it does divide, its specialized structure in great part disappears and it returns to a more embryonic condition. And we probably have in the cessation of the division of the trochoblasts simply an exhibition of this rule. The trochoblasts in *Planorbis*, after they have divided, become much flattened or thinned out. In eggs stained with silver nitrate they are almost transparent, while the cells of the cross are stained brown, causing this structure to appear in conspicuous contrast to the cells between the arms. With the growth of the trochoblasts the arms of the cross become more narrow, as if they were pressed together at the sides. They thus take a somewhat darker stain and stand out in a more conspicuous manner than before. The further history of the trochoblasts will be described in the section on the cell lineage of the prototroch.

Although only the anterior pair of trochoblasts in *Planorbis* enters into the formation of the prototroch, the term "trochoblasts" has been applied to the posterior pair as well. The latter form a portion of the head vesicle. As this structure may be considered as consisting mainly of the enlarged posterior portion of the prototroch, it would scarcely be incorrect to call these cells trochoblasts also.

The Division of the Third Quartette.—The cells of the third quartette are large and elongated in a meridional direction. Their cleavage is almost exactly radial, as in *Limax*. In *Crepidula*, according to Conklin, "the direction of the cleavage is nearly radial, though after the cleavage has occurred it is seen to be plainly laeotropic in $3a$, $3b$, and $3c$, and dextro-tropic in $3d$, i.e., the cleavage is nearly bilateral on the posterior end of the ovum." Yet the spindle in $3d$ is sometimes laeotropic, as Conklin adds, and the nuclei may show this relation to each other, while "the cell body may show reversal of cleavage." "This," to quote the same author again, "is but another illustration of the fact that bilaterality first appears on the posterior side of the egg, that it is due to the change in direction of one out of four cells, and that it is not perfect when it first appears, but is merely a deviation from the spiral type toward the bilateral." In *Planorbis* the two cells on the posterior side of the egg, $3a$ and $3d$, divide before the anterior ones, $3b$ and $3c$, but I have been unable to find any constant deviation from the radial direction of their cleavage. A radial cleavage may, however, be considered an approach toward the bilateral type, and we may view the earlier division of the cells $3a$ and $3d$ as the first foreshadowing of bilateral cleavage.

The lower cells of the third quartette are somewhat smaller than the upper ones and have their long axis horizontal, while the long axis of the upper cells is still radial. In *Neritina* and *Umbrella* the cleavage of the cells of this quartette is also nearly radial, and the lower cell is the smaller, as is also the case in *Crepidula*, and, according to Kofoed's and Meisenheimer's figures, in *Limax*. In *Physa*, according to Wierzejski, the cleavage is also meridional and unequal, but in the anterior

quadrants the smaller cell is nearer the animal pole, while on the posterior side of the egg the reverse is the case. Which of the cells are the first to divide is not stated. In another pulmonate, Siphonaria, the division of the posterior cells of the third quartette occurs before that of the anterior ones, each cell giving off a small cell toward the vegetal pole. The division in both cells is laeotropic, but less so in the cell on the left side (Fujita, '95, p. 91, Fig. 5, 6x). This cleavage, according to Fujita, occurs at the thirty-two-cell stage; the corresponding cells in the anterior quadrant have not divided at the stage in which the egg contains forty-three cells, which is as far as the cell lineage of this form is described.

The first division of the cells of the third quartette seems to mark the point in the cleavage of gasteropods where bilateral cleavage makes its first uncertain appearance. In no case is there a typical spiral cleavage of all the four cells. The cleavage may be radial (Physa, Limax, Planorbis, Neritina, Umbrella), slightly bilateral in the posterior quadrants, and spiral in the anterior ones (Crepidula), or spiral in the posterior quadrants, but with an approach toward the bilateral type (Siphonaria). The posterior quadrants generally divide before the anterior ones. In Limax, however, the order of cleavage in the different quadrants seems to be inconstant (Kofoid). In Crepidula, Umbrella, and Planorbis the posterior cells divide only a short time before the anterior ones, while there is a long interval between these divisions in Siphonaria. The cleavage of the cells of this quartette is, in all the above forms, unequal, and, except in the anterior quadrants in Physa, the smaller cell lies nearer the vegetal pole of the egg.

The Formation of the Primary Mesoblasts. — Soon after the twenty-four-cell stage is reached, the posterior macromere *D* divides into unequal parts. The upper moiety is much larger than the lower one, and, from the first, lies partly pushed into the cleavage cavity, so that only a small portion of it appears at the surface of the egg (Pl. XVII, Fig. 11). It is dark and granular, and contains a large amount of yolk, like the cells of the entoderm. The division of *D* is dextrotropic, as Crampton found it to be in Physa. The primary mesomere lies, therefore,

to the left of *D* in forms with reversed cleavage, and to the right of *D* in forms in which the cleavage is not reversed. Before the division of *D*, the two macromeres, *B* and *D*, were equal in size, and the anterior and posterior sides of the egg could not be distinguished. The position of *4d* or *M* enables one henceforth to easily locate the posterior side of the egg. The primary mesomere soon divides in a nearly horizontal direction, though the cleavage is slightly oblique. This division is completed, as in *Siphonaria*, before the other cells of the fourth quartette arise. The two mesomeres gradually sink further into the egg and lose connection with the surface at about the sixty-four-cell stage.

One cannot compare the formation of the primary mesoblast in the different pulmonates, in which its origin has been traced, without being struck with the very close similarity of the process in the several forms. The exact cell origin of the primary mesoblast has been determined in *Limax*, *Physa*, *Lymnaea*, *Planorbis*, and *Siphonaria*. In all these forms the mesomere arises, shortly after the twenty-four-cell stage is reached, by the oblique division of *D*; it lies, from the first, partly pushed into the cleavage cavity; it is much larger than the entomere *D*, and of an opaque appearance; and it soon divides in a nearly horizontal direction and gives rise to the mesoblastic bands. A similar origin of the primary mesoderm is found in several other gastropods, but, with the exception of *Umbrella*, the mesomere is considerably smaller than the entoderm cell *D* (*Bythinia*, *Crepidula*, *Neritina*, *Ilyanassa*, *Fulgur*). There are at present many divergent accounts of the origin of the mesoblast in the mollusca. These accounts have recently been reviewed by different writers on the subject (Heymons, '93, Tönniges, '96, Schmidt, '95, Meisenheimer, '96), and fully discussed in Korschelt and Heider's *Embryology*, so that it would be superfluous to devote space to the subject here. In almost every case, however, in which the cleavage has been followed with sufficient care, the primary mesoderm has been found to arise, as in the above forms, from the posterior macromere *D*. Yet careful study has failed to discover pole cells in *Paludina vivipera*, but the fate of the cell *4d* in this form has never been traced.

What becomes of this cell in this form would be a matter of considerable interest.

The Fourth Quartette.—The fourth quartette is produced by a dextrotropic cleavage of the entomeres. All the cells of this quartette are large and full of yolk; the four cells at the vegetal pole are small and clearer than the others. They are quite thin and, therefore, less in bulk than their superficial area would indicate. The general appearance of the entoderm cells at this stage is very similar to those of *Limax*, and markedly different from those of most marine forms, in which there is usually much yolk.

The number of cells in the egg when the fourth quartette is formed is forty-nine.

Cells of the first quartette	16	{ 8 trochoblasts
Cells of the second quartette	16	{ 8 apical cells
Cells of the third quartette	8	
Cells of the fourth quartette	5	{ 2 mesomeres
Cells at the vegetal pole	4	{ 3 entomeres

One of the most conspicuous features of the egg at this stage is a belt of large cells around the equator. It is composed of twelve cells, the four middle pairs of cells of the second quartette alternating with the four upper cells of the third. All of these cells are more or less oblong, with their long axes vertical. A similar belt of cells may be seen, though less plainly, in *Crepidula* and *Limax*.

The History of the Cross.

At the time of its first appearance the cross contains eight cells of the first and four cells of the second quartette. Its center is at the apical pole of the egg, and its arms are anterior, posterior, right, and left. The tips of the cross lie over the large entomeres of the fourth quartette, the cells of the second quartette lying between. The median plane of the egg would cut through the middle of the anterior and posterior arms, and the entomere 4*b*, and pass between the two mesoblasts. Previous to the formation of the fourth quartette, this plane would

have cut the ventral cross furrow at almost a right angle. After the fourth quartette is formed, owing to the shifting of the small cells at the vegetal pole, the cross furrow would be cut at an oblique angle. Viewed from the animal pole, the cross furrow has been turned several degrees in a laeotropic direction. This rotation is obviously a result of the dexiotropic cleavage of the macromeres. Of course, if we take the small cells at the vegetative pole as fixed, we might consider that the rest of the egg, the fourth quartette and the ectomeres, had rotated in an opposite, or dexiotropic, direction. In an egg like that of *Crepidula*, in which the homologues of these small cells form the greater part of the bulk of the egg, it is natural to regard the furrows between these cells as fixed, and to speak of a laeotropic rotation of the ectoblast rather than a dexiotropic rotation of *A*, *B*, *C*, and *D*. Whichever pole of the egg we regard as fixed,—and this is the essential point,—it is certain that the rotation in the two forms has taken place, in accordance with their reverse types of cleavage, in opposite directions.

The next cleavage in the cross occurs in the basal cells at about the sixty-four-cell stage. This division is nearly radial, though slightly laeotropic. It is an interesting fact that we have here a violation of the rule of alternation of spirals exactly where it first occurs in *Crepidula* and *Neritina*, but with this difference, that in *Planorbis* the two successive cleavages are laeotropic, while in *Crepidula* and *Neritina* they are dexiotropic.

The apical cells $1a^{1,1}$, $1b^{1,1}$, etc., next divide. There seems to be a tendency to a dexiotropic cleavage in these cells, but the direction of the division appears to be more or less inconstant. To the extent that this cleavage may be considered dexiotropic, it is in accordance with the rule of alternation of spirals. This division marks the close of the period of definite spiral cleavage in the cells of the cross. The subsequent cleavages, to which this may be considered a transition, are all of the bilateral type. With the completion of this division the number of cells in the egg reaches 104. In *Crepidula* this cleavage is laeotropic, and Heymons mentions the fact that in *Umbrella* these cells divide, but says nothing concerning the direction of the cleavage. The

next cleavage occurs in the basal cells of the arms of the cross, and takes place in a radial direction. Each arm of the cross now contains three cells, besides the tip cells, which belong to the second quartette. This division is in the same direction as the preceding division of the basal cells, so there occurs a second exception to the rule of alternation of spirals. It is a noteworthy fact, also, that the direction of this division is at right angles to the corresponding cleavage in *Crepidula*. In *Crepidula*, when there are three cells in each arm of the cross, the two cells behind the small tip cells are much elongated transversely to the long axis of the arms, and it is natural that their cleavage should be at right angles to their longest diameter. The middle cells of the arms in *Crepidula* divide first; this division is followed by the cleavage of the cells at the apical pole of the egg, and soon afterward the division of the basal cells takes place. In *Planorbis* it is the apical cells that first divide; then the basal cells divide radially, so that before the longitudinal splitting of the arms occurs each arm has one more cell than in *Crepidula*; a radial division, lengthening the arms in *Planorbis*, takes the place of a transverse division, splitting the arms in *Crepidula*. Moreover, the cells lying just behind the tip cells of the cross, which in *Crepidula* divide first, in *Planorbis*, except in the anterior arm of the cross, *never divide again*.

The inner median cell of the anterior arm of the cross next divides transversely and marks the beginning of the splitting of the arms of the cross. After a short interval the splitting of the lateral arms also begins; the cleavages are slightly oblique, the lines of division pointing toward the anterior end of the cross. This nearly transverse division occurs only in the basal cells and the ones lying next to them, the inner median cells. *The basal cell in the anterior arm undergoes no further divisions*. Its history will be described later. The transverse splitting in the anterior arm at this stage is, therefore, limited to a single cell. The posterior arm of the cross, as in *Crepidula*, remains undivided throughout its entire history. With the longitudinal division of the basal cell above described, the history of the cleavages in the posterior arm is completed.

The composition of the cross at this period is as follows (Pl. XX, Fig. 37): Four rather small cells in the center, $1a^{1.1.1}$, $1b^{1.1.1}$, etc., around the apical pole of the egg; around these are four other cells, the intermediate cells, $1a^{1.1.2}$, etc., in the angles between the arms; the anterior arm, which is broader than the others, consisting of a single basal cell, $1b^{1.2.1.1}$, then a pair of cells, $1b^{1.2.1.2.1}$, $1b^{1.2.1.2.2}$, resulting from the transverse division of the inner median cell; then a single outer median cell, $1b^{1.2.2}$, and finally the tip cell, $2b^{1.1}$; the two lateral arms consisting of two pairs of cells at the base, an undivided outer median cell, and a tip cell; the posterior arm, consisting of a row of four cells. In all, the number of cells in the cross is twenty-nine. The anterior arm is shorter than the others, and the tip cell is more or less clear. The arms of the cross are slightly oblique; and it is worthy of note that the direction of their inclination is laeotropic, while in *Crepidula* and *Ischnochiton* the arms show a slight dextrotropic twist. This difference is doubtless connected with reverse types of cleavage of these forms.

The tip cells of the arms have enlarged and become transparent. The tip cell of the right arm is widest behind, while that of the left is widest in front; the outer median cells show the reverse relation. The cells of the posterior arm of the cross are usually rhomboidal in outline. The posterior tip cell is large and elongated in the direction of the arm, while the long axis of the other tip cells is transverse to the arm. The anterior tip cell is the smallest of the four. As the tip cells in eggs stained with silver nitrate are clear, the portion of the cross which stains dark is that derived entirely from the first quartette. This portion is very conspicuous, as it is surrounded on all sides by transparent cells.

It may be well, before tracing the history of the cross further, to compare it briefly with the cross in other mollusks. Blochmann traces the history of the cross in *Neritina* to a stage in which each arm contained three cells, except the posterior arm, which was composed of four. Blochmann's probably incorrect derivation of the tip cells has already been mentioned, and Kofoed has shown also that it is almost certain that his deri-

vation of the basal cells of the cross was likewise incorrect. Interpreting Blochmann's figures in the light of what is known to be the rule in other forms, it is evident that the cell lineage of the cross in *Neritina*, at the stage when there are three cells in each arm, corresponds exactly to that of *Umbrella*, *Crepidula*, and *Planorbis*. In the lengthening of the posterior arm of the cross by the addition of another cell, there is a further point of agreement with the history of the cross in the last two forms. In *Crepidula* both the basal and the tip cells of the posterior arm of the cross divide radially at about the same time, so that this arm comes to have one cell more, instead of one less than the other three. Two of the four cells in this arm, therefore, belong to the first quartette and two to the second. Whether three of the four cells of the posterior arm in *Neritina* belong to the first quartette, or whether there are two cells each of the first and second quartette, is uncertain. Blochmann says nothing about the derivation of this additional cell. Should we argue from analogy with *Crepidula*, we should be led to accept Conklin's scheme of the probable derivation of this cell, and derive the two posterior cells from the second quartette. If, on the other hand, we should draw our conclusion from a comparison with *Planorbis*, we should infer that the three anterior cells belonged to the first quartette, while only the tip cell belonged to the second. In *Planorbis* the four cells in each arm have the same derivation and are produced at nearly the same time. In both *Neritina* and *Crepidula*, owing to a delay in the division of the basal cell, the posterior arm contains at first but two cells, while each of the other arms contains three; this stage is followed by a stage in which the posterior arm contains four cells, while the number in the other arms remains the same. The tip cell in the posterior arm in *Crepidula* is larger than those of the other arms and divides first, and in a different direction from the others; that is, radially instead of transversely. The divisions of the cross cells in *Planorbis* are such that they preserve the radial symmetry of the cross much longer than in *Crepidula* and *Neritina*. There is little difference, either in the time or direction of the cleavages, in the different arms until after the period in which each arm is composed

of over four cells. The radial symmetry of the cross is not destroyed until the egg contains over one hundred blastomeres. In *Neritina* the cross becomes a bilaterally symmetrical structure, by the lengthening of the posterior arm, when the egg contains only about fifty cells. In *Crepidula* the cross may be said to be bilaterally symmetrical from the very beginning of its formation, owing to the smaller size of the basal cell and the larger size of the tip cell in the posterior arm. At about the forty-eight-cell stage there are only two cells in the posterior arm and three in the others; the divisions which increase the number of cells in the posterior arm to four occur when the egg contains sixty-seven cells, at the same time that the first transverse division in the other arms is taking place. The appearance of bilateral symmetry in the cross in *Umbrella*, Heymons did not describe.

The striking differences in the history of the cross in *Planorbis* and *Crepidula* are most interesting. It is natural to seek for some explanations of the problems which these differences present to us. Why is it that the cleavage of the cell, which in *Crepidula* results in a splitting of the arm of the cross, produces in *Planorbis* a lengthening of the arm of the cross? Why does the posterior tip cell in *Crepidula* divide before the others, and in a different direction, while it does not divide at all in *Planorbis*? Why is the radial symmetry of the cross retained longer in *Planorbis* than in *Crepidula*? And what is the cause of the very different behavior of all of the tip cells in the two forms? These are a few questions which suggest themselves when we compare the history of the cross in these forms. A complete solution of these problems is at present impossible, but we may, perhaps, determine some of the proximate causes of these differences of behavior. The transverse division of the cells, $1a^{1,2,1}$, etc., in *Crepidula* is doubtless the result of the fact that the long axes of these cells are transverse to the arm of the cross. In *Planorbis* the basal cells in the arm, at the time they begin to divide, have their longitudinal axes in the contrary direction. Hence it is natural that their division should be radial and not transverse. Now the different shape of these cells in *Planorbis* is apparently due to the growth of the trochoblasts, which, as they increase in size, crowd the adjacent cells

together. Thus the arms of the cross would be subjected to a lateral pressure, which would tend to give the cells an elongation in a radial direction. In fact, as the trochoblasts increase in size, the arms of the cross actually do become narrower, as may readily be seen by comparing the figures of the earlier and later stages in the history of this structure. In *Crepidula* the trochoblasts are relatively much smaller than in *Planorbis*, and, at the time that the divisions above described occur, have not attained a sufficient size to exert much influence upon the arms of the cross. Thus the proximate cause of the difference in the direction of the division of the basal cells of the cross in the two forms seems very probably to lie in the different character of the trochoblasts. The cause of these differences in the trochoblasts may, as has been suggested above, lie partly, at least, in the relative size of the first quartette of ectomeres in the two forms, which, in turn, is largely dependent on the amount of yolk in the egg.

Are there any facts which throw any light on the different behavior of the cells in the posterior arm of the cross in the two forms? It will be remembered that in both *Crepidula* and *Planorbis* the posterior arm always remains undivided; *i.e.*, it consists of but a single row of cells. In *Crepidula* the development of this arm of the cross lags behind the others, the first basal cell not dividing until a considerable time after the others. Why is this? The explanation appears to lie in the fact that the cell $1d^{1,2}$ is smaller than the other three basals. Conklin, I believe, does not mention the fact, but in all of his figures this cell is uniformly represented as smaller than the others (Figs. 23, 25, 26, 29-31), in one case (Fig. 31) the difference being very marked. Further back than this it is to be noted that the division of the cell from which the basal cell $1d^{1,2}$ arose seems to be delayed, a fact which would indicate the smaller size of this cell, although this is not otherwise noticeable. It is certain, however this may be, that this cell $1d^1$ divides more unequally than the others, and the principal cause of the delayed cleavage of the posterior basal must, therefore, be sought in whatever agency gives rise to the unequal division of $1d^1$. In *Planorbis* the division of this cell is no more unequal

than that of the others in the same tier, and the basal cell of the cross which arises from it, being, therefore, of the same size as the other basals, divides approximately at the same time. We have, therefore, in the different character of the division of $1d^1$ in *Crepidula* and *Planorbis*, the beginning of the difference in the course of development of the posterior arm of the cross in the two forms. We may be unable to explain the differential character of this cleavage of $1d^1$. Why one out of four protoplasmic cells, identical, so far as we can determine, in size and structure, should divide much more unequally than the others is certainly not apparent. Nevertheless it is desirable to find the precise point where the histories of structures in different forms begin to diverge, although we are unable to discover the antecedent phenomena which give the direction to the different lines of divergence. The earlier cleavage of the posterior tip cell in *Crepidula* is probably connected with its larger size. Its cleavage is radial, and each of the daughter-cells divides again in a radial direction, giving rise in all to six cells in the posterior arm. The cause of these successive longitudinal divisions may possibly be the growth of the posterior trochoblasts and the cells lying below them, which at this period have reached a considerable size. The enlargement of these cells would naturally subject the arm to pressure at the sides and give the cells a longitudinal elongation. A comparison of Conklin's Fig. 49 with Fig. 53 shows that the trochoblasts in the latter figure are larger, and the basal and tip cells longer and narrower. The shape of the tip cells just before their division is not figured, but it is probable that they have become more or less compressed like the basals, after the stage shown in Fig. 51. However, after their division they have been narrowed to about one-half their former diameter (Fig. 53). The posterior tip cell in *Planorbis* has quite a different history. In the first place it is no larger than the tip cells of the other arms, but the marked difference it presents from the corresponding cells in *Crepidula* is that it never divides. It increases enormously in size and becomes transparent. Its shape changes entirely; at first it is elongated transversely to the arm of the cross; gradually, as it enlarges, it becomes

elongated in the opposite direction and takes part in the formation of the head vesicle. The elongation of this cell goes hand in hand with the growth of the posterior trochoblasts, whose increase in size would naturally subject it to a lateral pressure. The peculiar history of this cell is evidently correlated with the large size which the head vesicle attains in this form. It affords another case of precocious specialization of function such as occurs in the trochoblasts. In fact, the fate of this cell and the posterior trochoblasts is identical, as they all go to form the same organ. The head vesicle in *Planorbis* develops early, and reaches a much larger size than it attains in *Crepidula*. The posterior tip cell becomes differentiated at an earlier period and stops dividing. In *Crepidula* it divides twice, perhaps many times, and the products of these divisions remain for a long time apparently little modified. Their precise fate is uncertain; probably some of them, at least, enlarge and enter into the formation of the head vesicle, as in *Planorbis*.

The other tip cells divide twice in *Crepidula*, forming a row of four small cells lying across the tips of the arms of the cross. All of these cells, accordingly, go into the upper row of cells, forming the prototroch. Except in the anterior arm of the cross, the tip cells in *Planorbis* do not divide at all. The lateral tip cells, like the posterior one, enlarge, become transparent, and go to form a part of the head vesicle. There is probably no other group of cells in these two forms which present such marked differences of behavior as the tip cells of the cross. In *Crepidula* they are at first very small and of unequal size; they grow very little and divide several times. In *Planorbis* they are at first quite large and of equal size; they grow quite rapidly, and, with the exception of the anterior one, never divide at all. In *Crepidula* those of the anterior and lateral arms go into the prototroch; in *Planorbis* only the anterior one goes into the formation of this organ, and this cell, as far as could be determined, undergoes only one division.

The next cleavage, after the stage to which the history of the cross has been traced, occurs in the four cells lying in the angles between the arms. These divisions are bilateral; in the anterior pair of cells the left cell divides dextrotropically, the right

cell laeotropically; in the posterior pair the cleavage of the right cell is dextrotropic, that of the left one laeotropic. The two cells in the anterior arm, $1b^{1,2,1,2,1}$, $1b^{1,2,1,2,2}$, next divide, the right cell in a dextrotropic, the left in a laeotropic direction. The posterior cells on either side resulting from this division come to lie to the outside of the small, outer, intermediate cells, so that they no longer lie in contact with the trochoblasts (Pl. XX, Fig. 42). (Compare the cleavage of $1b^{2,2,1}$ and $1b^{2,2,2}$ in *Crepidula*.) Next the anterior median cell, $1b^{1,2,2}$, divides transversely, and each of the daughter-cells divides bilaterally, in the same direction as that of the pair of cells just described.

At this period the general form of the cross has undergone marked changes. The cells of the posterior arm have increased greatly in size and become distorted in shape. The posterior trochoblasts enlarge unequally, and the posterior arm may be pushed either to the right or the left. The posterior tip cell first increases in size; afterward, all the cells lying in front of it also enlarge. The enlargement of cells does not stop with the cells of the posterior arm, but the four apical cells increase in size also (Pl. XX, Fig. 42). The central and anterior portions of the cross are pushed forward more rapidly than the lateral arms, which thus appear to be bent backwards. The darkly staining portion of the cross has now the shape of a *V*, with the apex pointing anteriorly. The two anterior tip cells, as the cross is pushed forward, come to lie more and more nearly side by side, and, finally, they become arranged transversely across the tip of the cross. As they were originally somewhat oblique, this arrangement would very naturally result from a pressure due to the forward rotation of the apical cap of cells.

The process of enlargement of cells extends forward to the cells lying in front of the apicals, and eventually forms a tract of large clear cells, which separates the two halves of the cross and reaches the prototroch in front. The basal cell of the anterior arm of the cross, since it lies in the median line, takes part in this enlargement of cells; as it increases in size, it becomes pushed forward, and the cells in front of it, which lie symmetrically on either side of the median axis of the arm, are forced aside. The fate of this cell has been carefully traced, as

it has a peculiar and most interesting history. At first it lay at the base of the anterior arm of the cross. When the cells $1b^{1,2,1,2,1}$ and $1b^{1,2,1,2,2}$ divide, this cell comes to lie between the cells produced by these divisions, and soon it is seen further forward, between the posterior pair of cells arising from the third cell of the arm, $1b^{1,2,2}$. Pl. XX, Fig. 42, shows this cell where the anterior cells are in contact, and Pl. XX, Fig. 48, shows it pushed still further forward, until it has forced the cells $1b^{1,2,2,1,1}$ and $1b^{1,2,2,2,1}$ to either side and come into contact with the tip cells. Its journey does not end here, but it apparently pushes aside the tip cells as well, and comes in contact with the cells of the second quartette, which lie below them (Pl. XX, Fig. 46). *This cell has, therefore, pushed its way through the split anterior arm from the base to the tip.* In later stages it becomes much elongated transversely, and forms a part of the prototroch. The origin of this upper median cell of the prototroch was for a long time a puzzling problem. I have fortunately found all stages of the process by which it travels through the middle of the anterior arm of the cross to its definitive position.

The further history of the cells of the cross is very difficult to follow. The cells in the center enlarge unequally in different cases, and the position of the cells is altered considerably by this process, which makes it very difficult to follow their lineage. I have observed a bilateral division of the cells $1c^{1,2,1,1,1}$ and $1a^{1,2,1,1,1}$, the lines of division converging anteriorly. Both products of this division then divide at right angles to the preceding cleavage.

The Second Quartette.

The cleavage of the cells of the second quartette has already been traced to a stage in which there are four cells in each quadrant. The two middle cells in each quadrant, which are larger than the upper and lower cells, are the first to divide; the division of both cells in each pair is laeotropic, the cleavage of the right cell occurring a little before the left. These divisions occur between the fifty-two and the sixty-four cell

stages; the lower cells resulting from these divisions are somewhat larger than the upper ones. When the egg contains about seventy-five cells, the lower cell in each quadrant divides, generally in a laeotropic direction, but the direction of the cleavage does not appear to be constant. Pl. XIX, Fig. 32, shows that the division of $2a^{2.2}$ was probably dextrotropic, but shifting of the position of cells has of course to be allowed for. The cells $2a^{2.2}$, $2b^{2.2}$, and $2c^{2.2}$, previous to their division, have become more and more flattened in a direction transverse to the vertical axis of the egg. After their division their daughter-cells become flattened still more in the same direction; one of these usually lies above the other, which alone comes in contact with the entomeres; sometimes they lie obliquely, especially in the *a* and *c* quadrants, the outer cell touching the entomeres by a small portion of its boundary. These cells form the anterior and lateral boundaries of the blastopore, the cells of the third quartette lying at the angles. It will be convenient to apply the term "stomatoblasts" to these cells, $2a^{2.2}$, $2b^{2.2}$, and $2c^{2.2}$, and their derivatives, as they form a part of the margin of the blastopore, and later take part in the formation of the stomodaeum. The term "stomatoblasts," however, was used by Wilson to designate the cells $2a^2$, $2b^2$, and $2c^2$, and their derivatives, which in *Nereis* form a ring of cells around the blastopore. In *Planorbis* the cells $2a^{2.1}$, $2b^{2.1}$, and $2c^{2.1}$ never come in contact with the blastopore, and probably do not take part in the formation of the stomodaeum, so that the term "stomatoblasts" would hardly seem applicable to them. The term "stomatoblasts" is used, therefore, to designate only the lower product of the division of the cells which correspond to the stomatoblasts of Wilson. In *Nereis* the division of the cells $2a^2$, $2b^2$, and $2c^2$ is transverse to the polar axis and all of the products of the cleavage border on the blastopore. In *Planorbis* these cells divide in nearly the opposite direction, so that $2a^{2.1}$, $2b^{2.1}$, and $2c^{2.1}$ lie above the ventral cells and never come into relation with the entomeres. The same cells divide into an upper and a lower moiety in *Unio*, *Neritina*, *Umbrella*, *Crepidula*, *Limax*, and *Ischnochiton*. In none of these forms, except *Ischnochiton*, has the cleavage of $2a^{2.2}$, $2b^{2.2}$, $2c^{2.2}$ been described. The cell

$2d^{2,2}$ differs in shape and in the direction of its cleavage from the other cells of the same tier. Before it divides, it becomes elongated in a vertical instead of a horizontal direction. It divides laetotropically, its daughter-cells lying obliquely side by side, with their long axes nearly radial. Later, owing to the approach of the large cells $3a^2$ and $3d^2$, the cell $2d^{2,2,2}$ becomes pushed upwards, losing its connection with the entoderm when these cells meet in the middle line.

The next divisions in this quadrant occur in the cells $2a^{1,2,2}$, $2a^{2,1,2}$, etc. The direction of the cleavage is nearly radial, but slightly dextrotropic. The corresponding cleavages in the b quadrant are delayed until a later period. The upper pair of cells in the quadrants a , c , and d next divide, the right cell laetotropically, the left dextrotropically. The cells from the adjacent arms of the cross push under the trochoblasts from either side and often meet each other, thus separating the trochoblasts entirely from the cells of the third quartette (Pl. XIX, Fig. 32). Owing to the forward rotation of the apical cap of cells, the cells $2b^{1,2,1}$ and $2b^{2,1,1}$ become pushed apart, so that they come to lie on either side, instead of above the cells $2b^{1,2,2}$ and $2b^{2,1,2}$ (see Pl. XX, Fig. 39). The cleavage of the tip cell $2b^{1,1}$ has already been mentioned; its two daughter-cells, owing to the rotation of the apical pole, come to lie side by side and in contact with the cells $2b^{1,2,2}$ and $2b^{2,1,2}$. The cells $2b^{1,1,1}$, $2b^{1,1,2}$, $2b^{1,2,1}$, $2b^{2,1,1}$, $2b^{1,2,2}$, and $2b^{2,1,2}$ all become large and clear and enter into the formation of the prototroch. In the anterior quadrant the three upper cells of the four, therefore, enter into the prototroch as in the annelids, and their products undergo, I believe, no further divisions beyond the stage just described. The cell $2b^{2,2,1}$ divides horizontally; this is the last cleavage in this quadrant that could be observed. The cleavage of the second quartette has been followed to a stage in which there are eleven cells in each of the quadrants a , c , and d , and ten in the anterior, or b , quadrant. After this stage the divisions of this quartette become very difficult to follow. I have seen a nearly horizontal cleavage of $2c^{1,2,2,1}$, and have been able to recognize the cells of this quartette when there are fifteen cells in each quadrant, but only hypothetical derivations of these

cells can be given. The group of cells in the b quadrant of the second quartette is shorter and broader than the other groups. This is doubtless due to the forward rotation of the apical pole of the egg, which would exert a vertical pressure on this group of cells. The slower cleavage in this group may be also due, in a certain degree, to the same cause, though it is probably correlated with the fate of these cells. The portion of this quadrant which does not go into the prototroch forms only an exceedingly small portion of the body of the embryo, being used, as far as could be determined, to form a part of the stomodaeum.

The early cleavages of the second quartette in *Planorbis* are very similar to those of other gasteropods. Kofoed has traced the cleavage of this quartette in *Limax* to a stage in which there are four cells in each quadrant, and these cells agree almost exactly in relative size and arrangement with those in *Planorbis* at the same stage. Blochmann ('82) figures a stage in which there are seven cells of this quartette in each quadrant, and their arrangement is very similar to that found in *Planorbis*. Heymons has traced the lineage of this quartette to a stage in which there are eleven cells in each quadrant, but he does not describe the direction of the cleavage of the tip cells. Conklin has followed the cell lineage of the second quartette to a stage in which each quadrant contains eleven cells, and has traced the cleavage of the tip cells somewhat beyond this point. The agreement of the direction of the divisions of this quartette in *Planorbis* with those in the above forms is quite close, but there are some minor differences. In general, we may say that the cleavages are more nearly radial than in the two last forms. For instance, the cleavages of $2a^{1,2,2}$ in *Crepidula* and $2a^{1,2,2}$ in *Umbrella* are nearly horizontal, while in *Planorbis* they are nearly radial. All the cells lying between the tip cell and the lowest cell of the group have, at a stage when there are eleven cells in each quadrant, exactly the same lineage in *Umbrella*, *Crepidula*, and *Planorbis*. In the first two forms the cleavage of the tip cell has occurred, while the lowest cell, $2a^{2,2}$, $2b^{2,2}$, etc., remains undivided; in *Planorbis* the lowest cell has divided, while the tip cell, except in the anterior quadrant,

remains entire. In both Umbrella and Crepidula the divisions of the second quartette, as far as they have been traced, are, with the exception of the cleavage of the tip cell in the latter form (and, possibly, also in the former), perfectly similar in every quadrant. This may be due to the fact that the forward rotation of the apical pole occurs later in these forms. The fact that when this rotation is delayed the similar character of the division of the quadrants of the second quartette is maintained for a longer period, lends additional support to the view that in Planorbis the delayed cleavage in the anterior quadrant is causally connected with this rotation. The early beginning of this rotation in Planorbis, caused as it is by the more rapid growth of the posterior trochoblasts and the posterior arm of the cross, may be viewed as a result of the early formation of the head vesicle. This structure develops early in Planorbis, and reaches a larger size than in Crepidula or Umbrella. The different behavior of the cells of the anterior quadrant of the second quartette in these different gasteropods may thus be considered a sort of indirect effect of the different degrees of development which the head vesicle attains in these forms.

The Mesoblastic Bands.

The mesoblastic bands in Planorbis have been fully described by Rabl, who was the first to derive the mesoderm in the gasteropods from a single cell. A division occurs, however, after the first cleavage of the primary mesomere, which it seems that Rabl overlooked. After the two mesomeres have come to lie entirely in the cleavage cavity, each buds off at the anterior end a minute clear cell, which is often quite difficult to observe. The spindles are inclined slightly towards each other at their anterior ends, and the small cells that arise lie almost in contact with each other. The next cleavage of the mesomeres is horizontal and equal, and at right angles to the preceding division. There thus result an inner and an outer pair of large mesodermic cells, which are figured by Rabl in Figs. 17*a* and 17*b*. The inner pair of cells are the mesoblastic teloblasts. The next cleavage of the teloblasts is in the same direction as

before, but this time the division is unequal and gives rise to a small cell lying between the middle and outer cells. The next cleavage occurs in the outer pair of cells and in the same direction as the preceding division. In fact, all the divisions in the mesoblastic bands are henceforth in the same direction, until a considerably later period of development. At the time when there are three cells in each band the mesomeres form a concave row of cells in the posterior half of the egg. As the bands lengthen by teloblastic budding, they assume the shape of a horseshoe. Later they become resolved into scattered cells.

The Third Quartette and the Secondary Mesoblast.

At the stage in which the egg contains forty-nine cells the cells of the third quartette are eight in number, arranged in four vertical pairs, lying over the angles between the cells of the fourth quartette. The first cleavage of this quartette forms a transition from the spiral to the bilateral type, and the subsequent cleavages show a bilateral character in a more marked degree. At nearly the same time the *lower* pair of cells, $3b^2$, $3c^2$, in the two anterior quartettes, and the *upper* pair of cells, $3a^1$, $3d^1$, in the posterior quadrants, divide in a nearly horizontal direction into equal moieties. Later, at about the sixty-four-cell stage, the upper pair of cells in the anterior quadrants, $3b^1$, $3c^1$, divide in the same direction as the lower pair. The lower pair of cells in the two posterior quadrants, $3a^2$, $3d^2$, remain undivided until a much later stage. In each of the two anterior quadrants there are now two pairs of cells, the one pair lying directly above the other. In each of the posterior quadrants there is a pair of cells lying over a large undivided cell. It is easy to orient the egg from the lower pole at this stage by the bilateral arrangement of these cells. Definite spiral cleavage, which appears in such a marked way in the early divisions of the first and second quartettes, seems entirely absent in the third. The first cleavage is radial, and all the succeeding divisions appear to be bilaterally symmetrical with reference to the median plane of the future animal.

The next divisions in this quartette occur in the cells $3a^{1,1}$, $3a^{1,2}$, $3d^{1,1}$, $3d^{1,2}$, on the posterior side of the egg, and in $3b^{2,1}$, $3b^{2,2}$, $3c^{2,1}$, $3c^{2,2}$, on the anterior side. These divisions are radial and similar in character, each cell budding off a small cell toward the vegetal pole. There thus arise four pairs of small cells, the two anterior pairs lying in the angles between the entomeres on either side of the median plane, the two posterior pairs lying above the large cells, $3a^2$ and $3d^2$. The upper pair of cells in each of the four quartettes next divide in the same direction as before, forming a vertical series of four pairs of cells in the anterior quadrants, and a similar series of three pairs of cells above the large cells, $3a^2$ and $3d^2$, in the posterior quadrants. The third quartette now contains thirty cells, eight in each anterior quadrant, and seven in each posterior one.

The two pairs of cells, $3b^{2,1,1}$, $3b^{2,2,1}$, $3c^{2,1,1}$, and $3c^{2,2,1}$, become pushed in towards the cleavage cavity and become partly covered by the surrounding cells. They divide in a nearly horizontal direction, and their daughter-cells become pushed into the cleavage cavity still further. They form an irregular row of four small cells lying above the pairs of small cells in the angles between the entomeres. The period of their division is quite variable. In one case (Pl. XIX, Fig. 27) a division has evidently occurred in $3c^{2,2,1}$ before the upper cells have divided, but this does not usually occur. In Pl. XIX, Fig. 33, $3c^{2,2,1}$ has divided, while the cell lying beside it is entire, as are also the corresponding cells in the b quadrant. The lower products of the division of the uppermost cells in the anterior quadrants, $3b^{1,1,2}$, $3b^{1,2,2}$, $3c^{1,1,2}$, $3c^{1,2,2}$, divide in a nearly radial direction. Pl. XIX, Fig. 33, shows $3c^{1,1,2}$ dividing, while the cleavage in $3c^{1,2,2}$ is completed. The number of cells in each anterior quadrant is now twelve; the small cells in the angles between the entomeres have remained undivided since their origin; above this pair are the four cells, which are partly sunk into the cleavage cavity, arranged in a transverse row, and above these again are three pairs of cells in a vertical series. The number of cells in each posterior quadrant is still seven, and the whole number of cells in the third quartette is thirty-eight. The entire egg contains at this period about 150 cells. The four cells in each

of the anterior quadrants, which lie partly pushed into the cleavage cavity, finally lose connection with the ectodermic wall and come to lie in the blastocoel. The cells from the two sides nearly meet, forming a curved row of cells, the posterior ends of which nearly meet the anterior ends of the mesoblastic bands, which curve forward from the posterior side of the egg. The anterior row of cells forms what Wierzejski calls the secondary mesoderm. As far as can be judged from the very brief description of this process in Wierzejski's preliminary paper, the formation of the secondary mesoderm in *Physa* is very similar to, if not identical with, its formation in *Planorbis*. The first cleavage of the cells of the third quartette in *Physa* is radial, as in *Planorbis*, and the lower cell in the two anterior quadrants divides horizontally into a right and left cell. The cleavage of the upper cell is not described. The next division of the lower cells is the same as in *Planorbis*, each giving off a small cell toward the vegetal pole. The second pair of cells, $3b^{2.1.1}$, $3b^{2.2.1}$, $3c^{2.1.1}$, $3c^{2.2.1}$, divide again in a radial direction, the upper cells going to form mesoblast; the fate of the lower cells was not determined. In *Planorbis* this cleavage is nearly transverse, the outer cells being somewhat higher than the inner ones, and probably corresponding to the upper cells (Mutterzellen) in *Physa*. If the lower cells, $3b^{2.1.1.2}$ and $3b^{2.2.1.2}$, $3c^{2.1.1.2}$, $3c^{2.2.1.2}$, whose fate Wierzejski did not determine, also form secondary mesoblast, the cell origin of the secondary mesoblast in the two forms would be identical.

The last cleavages observed in the cells of the third quartette were those of the large cells, $3a^2$ and $3d^2$. These divide bilaterally and in a nearly horizontal direction, a small cell being given off from each at the outer end. Later these cells give off another small cell in the same direction as before. After these two divisions these cells are considerably reduced in size, but at the time gastrulation begins they form a rather conspicuous pair of cells, lying behind the nearly circular group of entomeres. The third quartette at this stage is composed of forty-two cells.

General Considerations on the Secondary Mesoblast.

The origin of the secondary mesoblast in the Mollusca is a subject to which, for many reasons, considerable interest is attached. We possess, however, at present very few accounts of the process by which the secondary mesoblast arises. The first case in which a double origin of the mesoderm has been carefully and accurately traced is that of *Unio*, studied by Dr. Lillie. In this form both the primary and secondary mesoblast are segregated at an early period. The primary mesoblast arises from $4d$, as in other mollusks; the secondary or "larval mesoblast," as it is called by Lillie, arises asymmetrically from a cell of the second quartette, $2a^2$, on the left side of the egg. This cell is gradually overgrown by the surrounding cells, and, after budding off two or three small cells to the surface, comes to lie entirely in the blastocoel. Although the secondary mesoblast arises asymmetrically, it afterwards becomes disposed in a symmetrical manner by the migration, apparently, of some of the cells to the opposite side of the egg. The larval mesoblast forms a kind of mesenchyme, which gives rise to certain larval structures, which disappear in later development, and its early segregation appears to be correlated with the early development and importance of these organs in larval life. It is a significant fact that the cell from which the larval mesoblast arises is larger than the corresponding cell on the other side of the egg.

Secondary mesoblast was discovered later in *Crepidula* by Conklin. It was found to arise near the edge of the blastopore in the three quadrants, a , b , and c , in which no other mesoblast was produced. The exact cell origin of this mesoblast Conklin was unable to trace, owing to the large number of cells in the egg at that stage, but, from the position of the mesoblast cells, it was shown to have arisen from the cells of the second quartette. There is an anterior mesoblast cell in the b quadrant, and a right and left cell bilaterally placed in the a and c quadrants. The d quadrant produces no secondary mesoblast unless at a very much later period. The mesoblast in *Crepidula* arises, therefore, in each of the four quadrants.

The secondary mesoblast in *Physa*, according to Wierzejski,

and in *Planorbis*, according to my own observations, has yet a different origin, arising from the cells of the third quartette in the two anterior quadrants. It arises, as in *Crepidula*, at a late period of cleavage, and its origin is likewise bilateral. As the cells of the third quartette are arranged symmetrically on either side of the median axis of the egg, its origin from three quadrants could not be bilaterally symmetrical. It is quite certain that, in *Planorbis* at least, no secondary mesoblast arises from the posterior quadrants, unless at a very much later period of development.

Several cases have been pointed out by Lillie, among accounts of the embryology of the lamellibranchs, where the figures of the authors show strong evidence of the existence of secondary mesoblast. The figures of *Cyclas* by Ziegler and Stauffacher, of *Teredo* by Hatschek, of *Anodonta* by Goette and by Schierholz, of *Ostrea* by Horst, show mesoblast cells in the early stages of gastrulation that could scarcely have arisen from the pole cells. In all these genera, cells are figured in front of, as well as behind, the blastopore. There are also similar cases in papers on other groups of mollusks. Kowalevsky's figure of a sagittal section of the larva of *Dentalium* shows a large mesoblast cell in the blastocoel at either end of the gastrula. And similar indications of secondary mesoblast are shown in Fol's figure of a sagittal section of the larva of *Firoloides*.

The case of *Paludina vivipera* is an interesting one in this connection. It is one of the few points of agreement, among those who have worked on the form, that mesoblastic pole cells do not occur. Tönniges finds that, in this form, mesoblast is produced from certain cells lying in front of the blastopore. If we accept Tönniges's account, the formation of the mesoblast in *Paludina* would seem to correspond to the formation of the secondary mesoblast in other forms.

The researches of Eisig and Wilson on the development of annelids suggest that the occurrence of secondary mesoblast may be typical for both annelids and mollusks, and indicate another striking point of agreement to the many which exist between the methods of cleavage of these groups. At the same time they serve to connect more closely the cleavage of anne-

lids and mollusks with that of the polyclades, in which the mesoderm has a radial origin from one or more quartettes of micromeres. Until more is known, however, of the origin, and especially of the fate, of the cells which have been called secondary mesoblasts, it must remain uncertain whether there is any true homology between these cells and the mesoblast of the polyclades, although such a comparison naturally suggests itself. The subject is one of considerable interest from the standpoint of phylogeny, and the reader may be referred for suggestive discussions of the problem to the papers of Conklin ('97), Wilson ('98), and Eisig ('98).

The Entomeres.

The egg of *Planorbis* is peculiar among the eggs of mollusks, in that the entomeres are of small size and undergo numerous divisions before the beginning of invagination. The fourth quartette consists of cells which greatly exceed in size the four small cells at the vegetal pole. The three cells of this quartette which form entoderm, *4a*, *4b*, and *4c*, divide horizontally when the egg contains about fifty-six cells. The six cells resulting from this cleavage are arranged in the form of a horseshoe about the four cells in the center, the opening between the ends of the curve being on the posterior side of the egg. The next cleavage occurs when the egg contains about ninety cells. Each of the six cells of the fourth quartette divides in a nearly radial direction. These divisions are, however, slightly oblique, and are bilaterally symmetrical with respect to the median plane of the egg. The divisions of the cells on the right side of the egg are slightly laeotropic, while those on the left side are slightly dexiotropic. These divisions are followed by a cleavage of the three small cells, *A*, *B*, and *C*, at the vegetal pole, forming a fifth quartette. The cell *D*, which is the smallest of the group, does not divide; nor have I been able to observe its cleavage at any subsequent stage, although it could be recognized after the process of gastrulation had made some progress. It is quite an exceptional fact that the cell *D*, which, in many forms, is the largest cell of the egg, should, in *Planorbis*, be the least in size

of all the cells. From the twenty-eight-cell stage until the period of gastrulation it has remained without a single division. The cleavage of the central cells is bilateral; the cell *B* divides radially; the cleavage of *A* is laeotropic; that of *C* dextrotropic. These divisions are followed by an equatorial cleavage of the cells of the fourth quartette. The derivatives of *4b* divide first, but the cleavage of the cells in the other two quadrants soon follows. There are, after these divisions are completed, twenty-four cells of the fourth quartette and three of the fifth; these, with the four small cells at the vegetal pole, make a total of thirty-one entomeres. The entoderm cells at this stage are of small and about equal size, and are easily distinguished from the surrounding ectoderm cells by their yellow color. Their number is increased somewhat before gastrulation has progressed very far, but I have not attempted to follow their cleavage beyond the point just described.

Small spheres of an albuminous substance gradually accumulate in the entoderm cells and become quite numerous near the period of gastrulation. These spheres stain very darkly in haematoxylin and make observation of the nuclei very difficult. At their first appearance, these dark bodies are found also in the ectoderm; but, as development proceeds, they become more and more confined to the entodermic cells. The staining reaction of these bodies is much like that of the surrounding albuminous matter in which the embryo floats, and it is very probable that they are simply masses of albuminous substance that has been ingested by the cells and has not been chemically transformed. After invagination certain of the entodermic cells increase enormously in size, becoming filled with a transparent, yellowish substance that is little affected by stains. Rabl has shown that in the end of the cells turned toward the enteron there are masses of deeply staining substances which he regards as material which has been absorbed by the cells, but not completely transformed into the yellowish substance which fills the greater part of the cell. It is doubtless the same material that forms the darkly staining bodies in the egg.

The ingestion of albumin occurs in a similar manner in the egg of *Limax*, and has been described by Meisenheimer, whose

figures show very clearly the history of the process. The deeply staining bodies occur in all the cells at an early period, even in the sixteen-cell stage, and increase in number as the development proceeds. The cells of the ectoderm take in the albumin more rapidly than in *Planorbis*; but, finally, this function is transferred entirely to the cells of the entoderm. The process of digestion of albumin, by which the egg is nourished and enabled to grow, is carried on at first with equal facility by all the cells of the egg. This process soon predominates in the entomeres, although, for a time, it is carried on more rapidly by all the cells. Finally, the ingestion of albumin is relegated to the cells of the enteron, which have become specialized for digestive purposes, and the other cells of the egg no longer share this function.

The Rudiments of the Cerebral Ganglia and Eyes.

The two portions of the cross, which are separated by the median apical plate, form the regions which give rise to the cerebral ganglia and eyes. These two rudiments, in eggs stained with silver nitrate, appear as dark masses surrounded on every side by large and very transparent cells. The tip cells of the lateral arms, and the cell lying immediately above them, do not enter into the formation of these masses, but increase in size and go into the head vesicle. With the exception of these two cells in each arm, all the cells in the lateral arms of the cross, the cells of the anterior arm, except the tip and basal cell, and the central region of the cross, except the four apicals and the two cells lying in front of them, enter into the formation of these two rudiments. The composition of these two patches of cells when they are definitely marked off may be seen in the following table :

LEFT RUDIMENT.		RIGHT RUDIMENT.	
$Ia^{1.1.2.1}$	$Ia^{1.2.1.1.1.2}$	$Ic^{1.1.2.2}$	$Ib^{1.2.2.1.1}$
$Ia^{1.1.2.2}$	$Ib^{1.1.2.2}$	$Ic^{1.2.1.1.2}$	$Ib^{1.2.2.1.2}$
$Ia^{1.2.1.1.2}$	$Ib^{1.2.1.2.2.1}$	$Ic^{1.2.1.2.1}$	$Ib^{1.2.1.2.1.1}$
$Ia^{1.2.1.2.1}$	$Ib^{1.2.1.2.2.2}$	$Ic^{1.2.1.2.2}$	$Ib^{1.2.1.2.1.2}$
$Ia^{1.2.1.2.2}$	$Ib^{1.2.2.2.1}$	$Ic^{1.2.1.1.1.1}$	$Ia^{1.1.2.1}$
$Ia^{1.2.1.1.1.1}$	$Ib^{1.2.2.2.2}$	$Ic^{1.2.1.1.1.2}$	$Ia^{1.1.2.2}$

There are the same number of cells in each of the two rudiments which, as far as the size and position of their component cells is concerned, show a perfect bilateral symmetry. Yet the derivation of the cells on the two sides does not exactly correspond. The right rudiment contains cells from three quadrants, while the left contains cells from but two. This is because the intermediate cells from the d quadrant lie to the right of the median axis of the egg. The posterior arm of the cross, which is composed of cells of this quadrant, takes no part in the formation of these structures. The two products of the cleavage of the intermediate cell and the apical $1d^{1,1,1}$, which enters the apical plate, are the only cells of the first quartette in the d quadrant which do not go to form the head vesicle.

The cells forming the cerebral rudiments multiply with great rapidity. The areas become thickened and a proliferation of cells occurs which gives rise to the cerebral ganglia. The early appearance of these rudiments was observed by Rabl, who designated them a bilobed apical plate (Scheitelplatte). The further history of the fundamentals of the cerebral ganglia may be followed in Rabl's paper. The areas above described are not exclusively employed in the formation of the cerebral ganglia and eyes. The cells become so numerous before differentiation begins that it is impossible to trace the cell origin of the different structures arising from them. As the eyes arise at the outer sides of the cerebral ganglia, it is quite certain that they are formed from cells derived from the lateral arms of the cross. Whether this can be said also of the tentacles is uncertain.

The Apical Plate.

The term "apical plate" has been applied by Conklin to a median belt of large, clear cells in *Crepidula* extending from the apical sense organ to the prototroch. It is composed of seven cells which become covered by fine cilia and remain for a long time undivided, while the neighboring cells rapidly multiply. In *Planorbis* there is a median belt of large cells extending from the head vesicle to the prototroch, and which, from its similarity to the apical plate in *Crepidula*, I have designated by

the same name. The peculiar apical sense organ in *Crepidula*, I am satisfied, does not occur in *Planorbis*. In the former genus the four cells which form this organ remain of small size, acquire a tuft of long cilia, and become united later with a pair of nerve cords which arise from the cerebral ganglia on either side. The existence of such a structure in a molluscan larva is a striking mark of relationship with the annelid trochophore. The presence of this organ in the larva of *Planorbis* might naturally be looked for, but the four apical cells which form this organ in *Crepidula* become in *Planorbis* very much enlarged and thinned out and form a part of the apical plate. The cells which compose this plate are six in number, the four apical cells just mentioned and a pair of cells lying in front of these. Of the origin of this pair of cells I am not entirely certain, but I think they are the cells $1b^{1.1.2.1}$ and $1c^{1.1.2.1}$. These cells arose from the divisions of the intermediate cells lying in the angles between the arms of the cross. The cell lineage of the apical plate in *Planorbis* may be expressed as follows:

$$\left\{ \begin{array}{l} \text{apical cells } 1a^{1.1.1}, 1b^{1.1.1}, 1c^{1.1.1}, 1d^{1.1.1} \\ \text{intermediate cells } 1b^{1.1.2.1}, 1c^{1.1.2.1} \end{array} \right.$$

The anterior end of the apical plate is limited by the upper median cell of the prototroch, and its posterior boundary is formed by the basal cell of the posterior arm of the cross.

The Cell Lineage of the Head Vesicle.

Owing to the fact that the cells composing the head vesicle in *Planorbis* are few in number and of large size, I have been able to determine the exact lineage of all the components of this structure. The head vesicle may be said to first appear when the posterior trochoblasts and the cells of the posterior arm of the cross begin to enlarge. This enlargement, which begins before the 100-cell stage, causes the upper pole to move toward the anterior side of the egg. The tip cells of the lateral arms of the cross enlarge rapidly, and, at a later period, the cells $1a^{1.2.2}$, $1c^{1.2.2}$, lying just above the tip cells, also enlarge. All of these cells, owing to the anterior rotation of the upper pole

of the egg, come to lie behind the masses of cells which form the rudiment of the cerebral ganglia.

The cells composing the head vesicle are given in the following table :

Cells of the posterior arm of the cross	$\left\{ \begin{array}{l} 1d^{1.2.1} \\ 1d^{1.2.1.2} \\ 1d^{1.2.2} \\ 2d^{1.1} \end{array} \right.$
Posterior trochoblasts	$\left\{ \begin{array}{l} 1d^{2.1} \\ 1d^{2.2} \\ 1a^{2.1} \\ 1a^{2.2} \end{array} \right.$
Cells of the lateral arms of the cross	$\left\{ \begin{array}{l} 1a^{1.2.2} \\ 2a^{1.1} \\ 1c^{1.2.2} \\ 2c^{1.1} \end{array} \right.$

The number of cells in the head vesicle is twelve, of which nine belong to the first and three to the second quartette. The area of the head vesicle, when it reaches its maximum size, is fully equal to that of the rest of the embryonic body. The cells composing it are very thin and transparent and of relatively enormous size. All of the cells which make up the head vesicle were present in the egg after the division of the cells $1a^{1.2}$, $1b^{1.2}$, etc., which occurs at about the 64-cell stage, and only one cell which is destined to form a part of the structure, *viz.*, $1d^{1.2.1}$, undergoes division after this period. The cells, indeed, become so exceedingly thin in later stages that it is difficult to see how their division could be effected. What becomes of these cells when the head vesicle disappears is uncertain.

The Cell Lineage of the Prototroch.

It has been long known that the velum in the pulmonate gastropods is a rudimentary structure. It was first noticed by Vogt, and has been described since in various pulmonates by Rabl, Fol, and Lankaster. The velar lobes, which are such a characteristic feature in the so-called veliger stage in other mollusks, are absent in the pulmonates, and all that can be said to correspond to the velum is a double row of ciliated cells extend-

ing from the ventral side of the body, immediately in front of the mouth, towards the dorsal side of the embryo. This row of cells has been called the prototroch, from its similarity to that structure in the annelid trochophore. Owing to the marked resemblance of this structure in the larvae of two such distinct groups as mollusks and annelids, considerable interest is naturally attached to a comparison of its cell origin in these forms.

The cell origin of the prototroch was first determined in the annelids by E. B. Wilson, who discovered that in *Nereis* the four cells, $1a^2$, $1b^2$, etc., which he called the trochoblasts, gave rise to this organ. Later, the origin of the prototroch in several annelids has been carefully studied by Dr. A. D. Mead, with the result that the cell origin of this organ was shown to be identical in every case. In *Amphitrite* and *Clymenella* the cell lineage of the prototroch was carried out in detail to a late stage, but the other forms studied agree with these as regards the protoblasts of this organ as far as their cleavage was observed. In both *Amphitrite* and *Clymenella* the prototroch arises from the four trochoblasts, which divide twice, forming sixteen cells, and three of the cells of the second quartette in each of the three quadrants, *a*, *b*, and *c*. Thus three out of the four cells of the second quartette in each of these three quadrants go to form the prototroch. Mead argues that in *Nereis*, also, the prototroch arises in the same way, although Wilson's derivation of this organ differs from Mead's as regards the fate of the upper cells of the second quartette. Child's account of the formation of the prototroch in *Arenicola* agrees, point for point, with Mead's, and Mr. Treadwell's observations in *Podarke* indicate a similar origin of the prototroch in that form (Child, '97; Treadwell, '97).

So far as known, the prototroch in the Mollusca arises in a manner which is strikingly similar to its origin in the annelids. Blochmann's derivation of the velum in *Neritina* from the peculiar granulated tip cells of the lateral arms of the cross is doubtless incomplete, since these cells form only a part of this organ in other mollusks, as in annelids. In *Crepidula* the velum at first consists of a double row of cells, the dorsal ends of which become "indistinguishable from the surrounding cells." "The

median portion of the first row," says Conklin, "arises from the cells which lie just beyond the ventral end of the apical plate. These cells are in all probability $1b^{1,2,2,1,2}$ and $1b^{1,2,2,2,2}$ [inserting the correction in Conklin's note, p. 204]. One of these cells is shown dividing in Fig. 71. In Fig. 72 a transverse row is formed, which is plainly the first row of velar cells." The portion of the first velar row lateral to these six cells is evidently derived from the anterior turret cells, $1a^2$ and $1b^2$. The anterior turret cells divide bilaterally in a nearly horizontal plane, and these, with the median cells just mentioned, form a row of cells, extending around the anterior half of the egg, connecting the tip cells of the lateral arms. The tip cells of the lateral arms divide, forming a transverse row of four cells, which forms a further continuation of the first row of velar cells as far as the undivided posterior trochoblasts and tip cells.

Regarding the second row, Conklin says: "It is probable that the mid-ventral portion of the second velar row, V^2 , is derived from the cell which I have identified provisionally as $2b^{2,2}$, and which lies just beyond the median cells of the first row (Figs. 56, 69, and 70). I have not been able to determine whether any part of the second row arises by subdivision of the cells of the first; if not, this row may include a few cells of the third quartette ($3a^{1,1,1}$ and $3b^{1,1,1}$, Fig. 56) at the points opposite the anterior turrets. . . . Thus the preoral velum is composed of a few cells of the first quartette, many of the second, and possibly a few of the third."

In *Planorbis* the cells composing the prototroch are few in number and are arranged in a double row. The products of the division of the tip cell of the anterior arm of the cross go to form, as in *Crepidula*, a part of the upper row of cells. The tip cell divides, as far as I can determine, but once, and the two daughter-cells become pushed apart by the cell $1b^{1,2,1,1}$, which forms the median cell of the upper row. These cells extend to the anterior trochoblasts on either side, but, in later stages, they may sometimes be separated from them by cells which wedge in from below. The anterior trochoblasts, which originally lay, the one above the other, became shifted by the anterior rotation of the upper pole of the egg, so that they come to

lie at nearly the same horizontal level, the originally upper cell lying in front. The tip cells of the lateral arms lie immediately behind the anterior trochoblasts, but do not, as I formerly supposed, form a part of the prototroch, but enter into the formation of the head vesicle. They mark, in fact, the transition between these two structures, and might be considered as greatly enlarged velar cells. The chief differences between the first row of velar cells in *Planorbis* and *Crepidula* are that in the former the lateral tip cells become greatly enlarged and do not divide, and the anterior tip cell divides but once, the daughter-cells becoming pushed apart by one of the cells of the first quartette.

The lower row of cells in the prototroch is derived from the second quartette. Conspicuous among these are the two large cells lying below the median portion of the upper row. They are symmetrically placed on either side the median line. The two cells originally lying above these, $2b^{1,2,1}$ and $2b^{2,1,1}$, have been forced aside by the forward rotation of the upper pole, so that the tip cells, $2b^{1,1,1}$ and $2b^{1,1,2}$, come to lie next to the cells $2b^{1,2,2}$ and $2b^{2,1,2}$. The cells of the lower row at the sides are small, and are added to the prototroch at a later stage, when the number of cells in the regions from which they arose was so great that it would be very difficult to trace their lineage.

The Shell Gland and the Foot.

The shell gland makes its appearance some time after the closure of the blastopore. A very early stage in the development of this structure is shown in Pl. XXI, Fig. 51. It is located a short distance behind the tip of the posterior arm of the cross, in a region which is formed from the cells of the second quartette. It is derived, doubtless, from derivatives of $2d^{1,2}$ and $2d^{2,1}$. It forms a tolerably deep invagination, the cavity of which becomes almost entirely obliterated.

The foot arises as a protuberance behind the mouth. The two halves are separated by a row of clear cells extending backward some distance from the definitive mouth — a fact which may or may not indicate a double origin of this organ. A very

similar group of cells is seen in the middle portion of the foot in *Crepidula* (Conklin, '97, p. 143). Both Lillie and Conklin derive the foot from cells of the second quartette. It seems probable that, in *Planorbis*, cells from the third quartette also enter into its formation. The cells immediately behind the blastopore are derived from the third quartette, and it is not unlikely that the median portion of the anterior end of the foot is derived from some of these cells.

The Larval Kidney.

The larval kidney in *Planorbis* has the form of a V-shaped tube, situated on either side of the body behind the head. At the junction of the two arms is a large perforated cell, the so-called giant cell, the lumen of which communicates with the canals of the arms. The inner arm is directed toward the head; it is formed of a row of perforated cells, and the lumen is ciliated and provided with a ciliated opening near the end. The outer arm is directed downward and is provided with an opening to the exterior. The movements of the cilia lining the arm of the larval kidney may easily be seen in the living embryo.

The larval or head kidney of the pulmonates has been observed several times by the older writers on the embryology of these forms (Stiebel, Ganin, Gegenbaur, Stepanoff), and it has been mistaken for the rudiment of the nervous system, and also for the oesophagus. The giant cell discovered by Bütschli rather strangely had escaped the notice of Fol, who has given an otherwise quite accurate description of this organ.

The development of the larval kidney of pulmonates has been studied by Fol, Rabl, and Wolfson, all of whom came to very different conclusions regarding its origin. According to Fol, the larval kidney in *Planorbis* makes its first appearance as a small ectodermal pouch. "La poche s'approfondit dans la direction du dos, de telle façon qu'elle finirait par rencontrer celle du côté opposé sur la ligne dorsale. Mais la croissance dans ce sens s'arrête de bonne heure, l'organe se recourbe à peu près à angle droit et s'allonge maintenant dans la direction de la bouche." The larval kidney, therefore, according to Fol,

is entirely of ectodermal origin. In a paper on the development of the fresh-water pulmonates, Rabl ('75) described this organ, which he mistook for a part of the nervous system, as arising in a somewhat similar way to that described by Fol.

According to Wolfson's account of the larval kidney in *Lymnaea*, a large velar cell on either side of the embryo is pushed inward, becomes perforated by a canal, and gives rise to a hollow outgrowth which is directed anteriorly and opens by a ciliated mouth. Wolfson holds, in opposition to Fol, that the Urniere are unicellular organs, basing his opinion on the study of a large number of sections.

The account of the origin of the larval kidney given in Rabl's later paper, "Ueber die Entwicklung der Tellerschnecke" ('79), differs radically from the foregoing descriptions. According to Rabl's later account, the origin of the larval kidneys can be traced back to a large cell lying in the mesoblastic bands. These large cells, according to Rabl, result from the first division of the mesoblastic teloblasts, though it is more probable they arose from the second (see p. 407). By the teloblastic budding of the two middle cells, two rows of smaller cells are produced which carry forward the large cells, v_1 , v_2 , at their ends. These large cells also bud off small cells anteriorly, like the teloblasts. Each mesoblastic band comes thus to consist of a large cell with a row of smaller cells in front, followed by another large cell with a similar row in front of it. The cells v_1 and v_2 become perforated by a canal and come to be elongated and bent, forming the giant cell of the larval kidney. Then some of the cells lying in front and behind the giant cells also become perforated and form the anterior and lower arms. The larval kidneys, therefore, according to Rabl, are multicellular organs and entirely mesodermic in origin. The teloblastic budding of the protoblasts of the larval kidneys is a fact of much interest. It recalls the behavior of the nephroblasts described by Whitman ('78) in *Clepsine*, only in *Planorbis* the two teloblastic series are placed end to end, instead of side by side. Such a difference might easily be produced by a variation in the direction of one of the divisions of the teloblasts.

In view of the contradictory accounts of the origin of the

head kidneys, and the important theoretic bearing of the subject, I was led to attempt to verify, if possible, Rabl's description of the development of these organs. I have not followed the subject in detail, but have carried it far enough to satisfy myself of the essential correctness of Rabl's results. The large cells, v_1 , v_2 , of Rabl are very conspicuous elements of the mesoblastic bands, especially in later stages, as they increase considerably in size. They may frequently be seen perforated by a canal; and one case was found in which the canal did not extend entirely through the cell, but was narrowed to a point near the posterior side. The shape of the canal would seem to indicate that it arose by a sort of invagination at the anterior end of the cell. It may be, however, that the perforation arises, as has been shown in other cases, by the coalescence of a series of intracellular vacuoles. The mesodermic origin of the giant cell of the head kidney is a matter about which, I believe, there cannot be any doubt. Whether a portion of the external canal is ectodermic in origin, as Erlanger ('92) found in *Bythinia*, is uncertain. The principal portion of the structure, however, undoubtedly arises from the mesoderm.

There can be little doubt, when we study the structure and development of the larval kidneys of the pulmonate gasteropods, that these organs represent true nephridia. As the definitive renal organs of the gasteropods are regarded as nephridia also, there occur in the pulmonates two pairs of these organs. Larval kidneys similar to those of the pulmonates have been observed in the embryos of *Oncidium* and some lamelli-branchs. There are sac-like mesodermic larval kidneys in *Paludina* and *Bythinia*, which may be the homologues of the above structures. Whether or not the two pairs of renal organs in *Nautilus* represent nephridial structures, it is quite probable that the existence of the two pairs of nephridia should be regarded as typical for the Mollusca. Whether this indicates that the molluscan body is composed of two segments, is a question which need not here be discussed.

Gastrulation; Fate of the Blastopore.

The beginning of invagination may be observed when the egg contains about 175 cells. The egg at this time is a hollow blastula with comparatively thin walls, especially on the upper side. The apical pole at this time has been pushed forward through an angle of about 45° , and the rotation continues during the process of gastrulation. For some time before invagination begins the egg flattens and the lower pole loses its convexity. The invagination is first seen in the center of the vegetal pole; the four central cells and the cells of the fifth quartette first sink in, forming a small, round concavity; as this pit becomes deeper, the cells of the fourth quartette are involved in the process. All of the yolk-laden cells become invaginated, and the stomatoblasts may be seen at the edges of the depression. Doubt has been expressed as to whether the cells called entodermic and ectodermic in the early cleavage of mollusks really prove to be so by their history. In *Planorbis*, at least, the edge of the blastopore marks quite sharply the boundary between the protoplasmic and yolk-laden cells. The invaginated area is at first nearly circular in outline, but, as the depression deepens, it assumes an elongated form; and, finally, the mouth of the gastrula becomes reduced to a narrow, slit-like orifice. The length of the elongated blastopore becomes reduced by closure from behind. The anterior end of the blastopore is a comparatively fixed point, and is situated just behind a pair of small cells of the second quartette. Pl. XX, Fig. 46, shows a stage in which the blastopore is reduced to a minute slit lying between two cells. Another preparation showed these two cells in contact, so that the blastopore in this species may be said to close, though it is only for a brief period. The oesophageal invagination occurs very soon after the blastopore closes, *and at exactly the same place*. Its orifice is small and nearly circular, and becomes surrounded by cilia. It gradually deepens and gives rise to a diverticulum on the ventral side, which becomes the pouch of the radula.

The gastrulation in *Planorbis* is purely embolic. The cells of the ectoderm do not slip over the edges of the entomeres, as

in many other gasteropods, even in the least degree. In the species of *Planorbis* studied by Rabl the blastopore is said to become the mouth. Whether this difference is merely a specific one, or whether Rabl failed to observe the blastopore during the short time it is closed, is uncertain.

PART II. GENERAL CONSIDERATIONS.

Reversal of Cleavage and Reversed Asymmetry.

The fact that in certain gasteropods, with sinistral or reversed shells, the cleavage is also reversed, was first pointed out by Mr. Crampton ('94). Crampton studied the cleavage of two closely related genera of fresh-water pulmonates, *Physa* and *Lymnaea*, the former of which has a sinistral shell, while in the latter the shell is of the normal or dextral type. The cleavage of *Physa* was found to agree, point for point, up to the stage at which the primary mesoblast is formed, with that of *Lymnaea*; but with this exception, that the direction of every cleavage is reversed. The reversal was observed in the cleavage which led to the four-cell stage, and was shown to give rise to a different position of the cross furrow in the two forms. Crampton also points out that *Planorbis*, according to Rabl's paper, affords another instance of reversed cleavage. The possibility of a causal connection between reversed cleavage and a reversal of the shell was pointed out, but further discussion of the subject was not attempted. Haddon's figure of the eight-cell stage of *Janthina* ('82) apparently shows, as Crampton observes, that the cleavage of this form is reversed. This would form the only exception to the rule that the cleavage is dextrotropic in all unreversed forms. It seems not unlikely, however, that Haddon's figure is misleading on this point.

The cleavage of another species of *Physa*, *P. fontinalis*, was found to be reversed by Wierzejski, and Brooks ('79) figures a four-cell stage of *Planorbis parvus*, which, according to his statement concerning the origin of the two upper cells, affords another instance of reversal of cleavage. There are, therefore, two species of *Physa* and three of *Planorbis* in which the cleav-

age is known to be reversed.¹ Planorbis is not usually described, however, as having a sinistral shell; in fact, the shell may be markedly dextral. In the celebrated series of Planorbis shells found in the deposits at Stannheim there is every gradation between shells which are coiled in one plane and shells which are as markedly dextral as those of Littorina or Paludina. Many recent species, *P. albus*, *complanatus*, and *nitidus*, for example, have a shell with a more or less decided dextral coil. Nevertheless Planorbis is a reversed form, whatever the direction of the coil of its shell may be. The anal and genital orifices and the opening of the mantle cavity lie on the left instead of the right side. This is the essential point; the direction of the coil of the shell is a secondary matter. The shell of Planorbis belongs to the type which Lang calls "pseudo-dextral," and has no necessary connection with the essential features of the asymmetry of the animal.

The fact that in five cases a reversed asymmetry of the animal is associated with a reversal of cleavage, while in all dextral forms whose cleavage is known with any degree of accuracy the cleavage is dextrotropic or unreversed, certainly affords a strong presumption in favor of the view that there is some causal relation between the nature of the asymmetry of the body and the type of cleavage of the egg. And there are facts which render this conclusion more or less probable *a priori*. Conklin found that the beginning of asymmetry in Crepidula could be traced back to the cleavage of a single entodermic cell. The time and direction of the cleavage of this cell were found to give the initial bending of the entodermic area in a direction which determined the direction of the coil of the embryo. If we suppose a reversal of cleavage to occur in Crepidula, leading to a corresponding division on the other side of the body, it is not improbable that there would result a reversal of the asymmetry of the animal.

An interesting case in relation to this problem is afforded by the "inverse embryos" of *Ascaris*, described by Zur Strassen ('96). In the normal embryos of *Ascaris* certain cells are

¹ I have recently found reversed cleavage in another sinistral gasteropod, *Ancylus rivularis* Say. (See the *American Naturalist*, November, 1899.)

arranged in an asymmetrical but perfectly definite manner; but Zur Strassen found that in exceptional cases — in one egg out of about forty — this asymmetry was reversed; that is, the arrangement of cells, normally occurring on one side of the embryo, was found on the opposite side, one embryo being the mirrored image of the other. As the adult *Ascaris* is, in certain respects, asymmetrical, Zur Strassen was led to ascertain the proportion of reversed specimens in this form. It was found that, out of 125 individuals, four were reversed. Reversed adults occur, therefore, in about the same proportion as reversed eggs. As reversed eggs were seen, even in advanced stages of development, in an apparently normal condition, Zur Strassen concludes that they develop into reversed adults. We have here a reversal of cleavage occurring as an exceptional variation in eggs of the same species and probably giving rise to a reversal of some features of the structure of the adult form.

In *Planorbis* I have been unable to trace the origin of asymmetry to the cleavage of a single entodermic cell, as Conklin did in *Crepidula*. Immediately before gastrulation begins the entoderm is composed of a nearly circular patch of small entomeres, which are quite numerous (over 30), and of nearly equal size. I am quite certain that, before invagination, the entoderm gives no hint of the direction of the coil of the adult animal, nor does it manifest any appreciable asymmetry until a long time after the gastrula stage. In the gastrula shown in Pl. XX, Fig. 46, the bilateral symmetry is almost perfect. This gastrula contains several hundred cells; yet, if one carefully examines the figure, which is an exact camera drawing showing the outline of every cell, it will be found that for nearly every cell on one side of the body a corresponding cell can be found on the other side. The only deviation from bilateral symmetry that could be observed in this gastrula was a slight torsion in the cells of the head vesicle. The torsion can be observed in most gastrulae, but whether it has any connection with the final asymmetry of the animal could not be ascertained. Attention has been called to the fact that the arms of the cross exhibit a slight twist in a laeotropic direction, and that the direction of the twist is doubtless connected with the reversed cleavage of

this form. This twist is slight, and can no longer be detected when the cross comes to be mainly resolved into two isolated patches of small cells. It is possible that the torsion of the cross is never really lost, and that it is in some way connected with the beginning of the reversed asymmetry of the animal. If this were true, the reversed asymmetry of the adult would be shown to be connected with the reversal of cleavage of the egg. The egg, however, passes through stages of development in which it exhibits a well-nigh perfect bilateral symmetry, so that it seems scarcely possible to connect directly these two phenomena. There must, however, be some structural basis for the asymmetry of the adult in the stages which exhibit such marked bilateral symmetry. The effects of reversed cleavage may be seen in the asymmetrical arrangement of certain cells forming the cross in the first stages of gastrulation. It does not seem improbable that this asymmetry persists in stages in which it can no longer be observed, and that it forms the structural basis of the reversed asymmetry of the adult.

The Relation between Reversed Cleavage and the Direction of the First Cleavage Plane.

If we compare the four-cell stages of the eggs of Lymnaea and Planorbis in respect to the relation of the first cleavage plane to the median axis of the future animal, the fact will become manifest that, in the two cases, this median axis is cut by the first cleavage plane at a different angle. In Planorbis and Physa the first cleavage plane makes with the median axis a negative angle of about 45° ; while in Lymnaea it cuts this axis so as to form with it a positive angle of 45° . It is obvious that, while in both cases the first cleavage plane is oblique to the median axis of the future animal, the first cleavage plane in the sinistral form is at right angles to this plane in the dextral form. As the second cleavage plane is always at right angles to the first, is it not reasonable to suppose that, in the sinistral gasteropods, the first cleavage furrow corresponds with the second in the other forms? Has there not been, in the reversed forms, simply a reversal of the order in which the first two

cleavage planes make their appearance? And is not this the circumstance that determines the different direction of spiral cleavage in the reversed gasteropods?

It has been discovered by Conklin that the first cleavage in *Crepidula* is prospectively spiral and dextrotropic, as indicated by the rotation of the nuclei in the two-cell stage from left to right. I have not convinced myself that there is an opposite rotation of the nuclei in the two-cell stage of *Planorbis*, but the second cleavage is clearly dextrotropic even in its first stages. There is doubtless some structural basis for the different character of the second cleavage in *Planorbis* in the two-cell stage. The second cleavage of *Crepidula* is laeotropic, and that of *Planorbis* dextrotropic; and it seems probable that this difference is connected with the different relations of the first cleavage to the future longitudinal axis of the animal. If we suppose that in *Crepidula* a preformed longitudinal axis is cut obliquely by the first cleavage plane, it may help us to account for the rotation of the nuclei in the two-cell stage, and consequently the laeotropic second cleavage. As this axis in the sinistral forms would be cut at the opposite angle, we may have, in this circumstance, an explanation of the different direction of the spiral cleavage that is manifested in the second, if not somehow in the first division of the ovum. Certain it is that, if the longitudinal axis of the embryo is in any sense preformed in the egg, it is cut at planes approximately at right angles to each other in the dextral and sinistral forms. An oblique cleavage in relation to the bilateral organization of the egg might cause a certain amount of torsion that would manifest itself either at the end of that division or at the beginning of the next. Given two eggs in which this bilateral organization is cut at opposite angles by the first cleavage plane, it is probable that the torsion in the two cases would take place in opposite directions.

General Considerations on Spiral Cleavage.

If we glance over the literature on spiral cleavage, we shall find that, in different forms, the first cleavage is said to stand in different relations to the future longitudinal axis of the ani-

mal. In most cases the first cleavage plane has been found to be oblique to this axis, and in many forms the median axis is found to lie approximately midway between the first two cleavage planes (Neritina, Physa, Planorbis, Clepsine, Discocelis). In some instances, however, the first cleavage plane is said to be transverse to the median axis of the embryo (Nereis, Umbrella, Teredo). The instances in which the first cleavage plane is said to be oblique are by far the most numerous, and it will be well to devote some attention to the purported exceptions to this rule, with a view to determine whether these exceptions are not more apparent than real. The first cleavage furrow, in advanced stages of cleavage, comes to follow a very crooked path, and the upper portion may run in a quite different direction from the lower. Since the entomeres are usually of large size, the portion of the first cleavage furrow lying between these cells has generally been taken to indicate the direction of the first plane of cleavage, while the upper part of this furrow lying between the ectomeres has been disregarded. It is this circumstance, as will appear, that gives rise to the different accounts of the axial relations of the first cleavage plane. *In the ectodermic portion of the egg the axial relations of the first two cleavage planes are remarkably constant in all forms with spiral cleavage.* The different quartettes of ectomeres have exactly the same relative arrangement in annelids, mollusks, and polyclades; and cells of the same origin in these forms have almost identical axial relations. In both annelids and mollusks certain cells become arranged in the form of a cross, the arms of which have very constant relations to the embryonic axes. The arms of the cross in mollusks, which are always made up of cells of similar origin, are always anterior, posterior, right and left. In the annelids the cross is mainly formed of cells corresponding to those lying between the arms of the molluscan cross, and the arms lie at an angle of about 45° with the median axis of the embryo. As the upper portions of the first two cleavage furrows pass between cells of corresponding origin in all these forms, they maintain almost as constant axial relations as the arms of the cross themselves. *It may be said that the longitudinal axis typically bisects the angle between the upper*

portions of the first two cleavage furrows. As Conklin observes, "no exception is known, either among mollusks or annelids, to the rule that the second and fourth quartettes lie in the future median and transverse planes, and that the first, third, and fifth quartettes lie midway between these planes. The axial differences, therefore, of the first two cleavage planes, which have been mentioned, are differences merely in the axial relations of the four primary entoderm cells, and do not affect the axial relations of the other cells of the ovum, which are always the same among annelids and mollusks."

The behavior of the first cleavage furrows in *Crepidula* is very instructive, for it proves that the coincidence of the lower portion of the first cleavage furrow with the transverse axis is not indicative of the axial relations of the first cleavage plane *at the time of its formation.* In *Crepidula*, before the formation of the fourth quartette, the two parts of the first cleavage furrow lie approximately in the same plane. Now the longitudinal axis of the future embryo is definitely marked out at this period by the direction of the anterior and posterior arms of the cross. The first and second cleavage planes at this stage are oblique to these arms of the cross, *and hence to the future longitudinal axis of the embryo.* Thus, as far as can be ascertained, the *original* direction of the first cleavage plane is oblique; subsequent shiftings of the cleavage furrows are of no concern. It is to be noted that it is the upper parts of the first two furrows that retain very nearly their original direction in relation to the median axis, while the axial relations of the lower portions change. The shifting of the lower portions of the first cleavage furrows in no wise alters the fact that the original direction of the first two cleavage planes is oblique. The transverse direction finally taken by the lower portion of the first cleavage furrow in *Crepidula* is, therefore, due to the fact that it has become shifted in relation to the future median axis of the embryo.

In *Nereis*, according to Wilson, the first cleavage plane is transverse, and the second coincides, approximately, with the future median plane. It is evident, as Mead has pointed out ('97, p. 301), that Wilson uses only the portion of the first

cleavage plane lying between the entomeres as the basis of orientation. In *Amphitrite*, according to Mead, "if the second cleavage furrow is followed around the whole egg, its course is found to be an irregular zigzag, but its general direction is at a considerable angle to the sagittal plane." In both *Nereis* and *Amphitrite* the portions of the first two cleavage furrows lying between the ectomeres have essentially the same axial relations. It is only in the portions of these furrows lying between the entomeres that the axial relations vary to any marked degree, and it is probable that in *Nereis*, as in *Crepidula*, the transverse direction of the lower portion of the first cleavage furrow is due to the shifting of the entomeres in relation to the median axis.

It is very probable that if the lower portion of the first cleavage furrow were not shifted in relation to the upper, the whole cleavage plane would be, in every case, oblique to the future median axis of the embryo. In fact, it may be said that, *when- ever the lower portion of the first cleavage furrow has a direction different from that of the upper portion, its direction has been altered in relation to a part of this furrow which lies at a remarkably constant angle to the future median axis of the embryo; this is consequently tantamount to becoming shifted in relation to the median axis itself.* In such cases the lower portion of the first cleavage furrow can no longer be considered indicative of the direction of the first cleavage plane.

It is apparently, therefore, a universal characteristic of forms with spiral cleavage that the first cleavage plane is oblique to the future longitudinal axis of the embryo. As far as is known, the first cleavage plane, in forms with normal or unreversed cleavage, always makes a positive angle with the future median axis, while it makes a negative angle with this axis in forms whose cleavage is of the reversed or sinistral type. These facts, I believe, are not devoid of significance in relation to the general theory of spiral cleavage. In the previous section the suggestion was made that the difference in the direction of the first spiral cleavages in *Crepidula* and *Planorbis* might be due to the circumstance that the first cleavage plane cuts a pre-formed longitudinal axis in one form at a positive, and in the

other at a negative, angle. We are naturally led from this supposition to the view that spiral cleavage, in general, may be due to the oblique direction of the first cleavage in relation to a preformed longitudinal axis of the ovum. The possibility is open, on the other hand, that the direction of the first cleavage is not predetermined in the ovum, and that the median axis is determined only during cleavage. The evidence, however, is apparently against such a view, although it is still far from complete. The fact that the isolated blastomeres of the gastropod egg exhibit the phenomenon of "partial development" (Crampton, '96) in a marked degree affords a very strong reason for regarding the median axis of the embryo as determined at the time of the first division. It does not necessarily follow that this conclusion is to be extended to all forms with determinate spiral cleavage; yet Crampton's experiments may rightly be held, I think, as affording no little support to this generalization.

If the first division were always of a spiral character, as it is in *Crepidula*, the second cleavage would, as a consequence, be also more or less oblique, and the spiral character of the succeeding cleavages may be regarded as simply a consequence of Sach's law, that the direction of every cell division tends to be at right angles to the preceding division. The second division in forms with determinate spiral cleavage is, I believe, always a spiral one. In *Planorbis*, as in *Physa* (Crampton, '94), *Limax* (Kofoid, '95), *Crepidula* (Conklin, '97), and *Amphitrite* (Mead, '97), the spiral character of this cleavage is manifested by the inclination of the spindles before a very marked constriction of the cytoplasm takes place, and cannot, therefore, be regarded as a consequence of the shifting of the blastomeres. It seems not improbable that the spiral character of the second cleavage is a consequence of a spiral tendency of the first cleavage — that the agencies that cause the rotation of the nuclei and spheres in *Crepidula* after the first division are present in other eggs also, although they produce no visible effect until the second cleavage. In both annelids and mollusks, spiral cleavage is soon superseded by cleavage of the bilateral type, and there appears no reason to doubt that bilateral cleavage in

these forms is correlated with the bilaterality of the future animal. May not the delayed appearance of bilaterality be due to the circumstance that the oblique direction of the first cleavage started the divisions in a spiral direction, which is only overcome later by the tendency to bilateral cleavage?

It is worthy of note, in this connection, that in many eggs the first cleavage plane is oblique to the axis of elongation. This is the case, according to Conklin, in *Urosalpinx*, and, according to Fol ('75), the first cleavage furrow in *Cymbulia* is oblique to the line connecting the two attraction spheres. Among the Rotifera, Tessin ('86) found that in *Eosphora* the first cleavage furrow is oblique to the long axis of the egg and, therefore, to the median axis of the animal. In *Callidina*, according to Zelinka ('91), the first cleavage plane is oblique to the long axis of the egg, but by a shifting of the cells it comes, finally, to be transverse. And in *Asplanchna*, Jennings found that the first cleavage amphiasier "lies at first somewhat oblique to the longitudinal axis of the egg, but before cleavage takes place the spindle swings into coincidence with it." The nucleus in the larger of the two cells subsequently rotates to the right, so that a line joining the two nuclei would cut the first cleavage plane at an oblique angle. The possibility suggests itself that the rotation of this nucleus may be due to the same circumstances that caused the oblique direction of spindle before cleavage. The behavior of the egg of *Asplanchna* would seem to indicate that it tends to divide obliquely, as in *Callidina* and *Eosphora*, but that this tendency is overcome by the tendency to divide at right angles to its longest diameter, and only manifests itself at the beginning and at the end of cleavage. The first cleavage of *Asplanchna* recalls that of *Crepidula* in that the phenomenon of nuclear rotation is manifested after the completion of division. It is quite evident that among these rotifers other factors besides the shape of the egg influence the direction of the first cleavage. According to Hertwig's law, it would be expected that the first cleavage plane would uniformly lie at right angles to the long axis of the egg. The fact that the first cleavage is oblique to this axis in *Callidina* and *Eosphora*, and manifests a tendency to obliquity in

Asplanchna, points to the conclusion that the egg possesses a certain degree of cytoplasmic organization, which tends to determine the cleavage in a certain direction, even in opposition to Hertwig's law. And this naturally leads one to suspect that the first cleavage plane may be predetermined in eggs like those of most mollusks and annelids in which no means of orienting it are apparent.

The observations of Blochmann ('82) on *Neritina* show, if correct, that in this form it is very probable that the longitudinal axis of the embryo is determined before the first division. Blochmann found that in the two-cell stage the granules which later go into the tip cells of the lateral arms of the cross were aggregated into two small patches on the two sides of the egg. If these groups of granules existed, as seems very probable, before the first cleavage, it would show that the axial relations of the embryo were determined in the undivided egg. "Aus dem Umstand," says Blochmann, "dass die hellen Körnchen schon auf dem Stadium der Zweitheilung vorhanden sind (Fig. 39), kann man wohl folgern, dass die beiden Anhäufungen auch schon in dem eben in die Furchung eingehenden Ei vorhanden waren, und dass sie die Enden eines Durchmessers einnahmen, also eine Achse bestimmten." And Blochmann adds, in a footnote: "Bei unbefruchteten Eiern habe ich manchmal nach dem Austritt der Richtungsbläschen um den animalen Pol eine Anhäufung von solchen hellen Körnchen beobachten können (Fig. 23 bis 26), wage jedoch vor der Hand nicht zu entscheiden, ob dieselben hierher zu beziehen sind." It is unfortunate that Blochmann's observations on this latter point were not more decisive. Could the chief axes of the ovum be determined before fertilization, it would manifestly exclude the possibility that the entrance path of the spermatozoön determines the longitudinal axis of the future embryo. If in any eggs with determinate spiral cleavage the direction of the first cleavage plane and, consequently, the median axis of the future embryo is determined only after fertilization, as is claimed by Mead in the case of *Chaetopterus*, it may still be true that the spiral character of the first cleavages is determined by a bilateral organization of the egg substance, which is developed between

the period of fertilization and the occurrence of the first division of the ovum. The direction of the first cleavage plane and the direction of the spiral tendency of the first cleavage (whether to the right or the left) are two different things and may well be determined at different times. Roux has discovered that, in the frog's egg, the plane of inclination of the egg axis, as well as the direction of the first cleavage, is determined by the entrance path of the spermatozoön. Although in *Rana esculenta* the axis of the eggs is oblique before fertilization, it was found by Roux that the inclination of the primitive egg axis stands in no constant relation to inclination of the axis of the fertilized egg, which is determined by the entrance of the sperm. There is thus in the frog's egg a redistribution of some of the egg substances after fertilization and before the first cleavage, with reference to the future plane of symmetry, if there be not a differentiation also of the cytoplasm. That the period between fertilization and cleavage is a period of active differentiation in the eggs of many forms is a conclusion to which many facts point. Further investigations will have to be made, however, before it can with certainty be determined whether, in eggs with spiral cleavage, the chief axes are established before the first division of the ovum by any sort of differentiation of the egg substance.

In eggs with bilateral cleavage it is the rule that the first cleavage plane and the median axis of the embryo coincide. There are several exceptions to this rule, however, but they occur in forms in which the cleavage is not of a highly determinate type, as is shown by the variations in the cleavage of different eggs of the same form. Where the cleavage is definite and determinate, the cell divisions occurring with regularity and precision, with little individual variation in the eggs of the same species, the direction of the first cleavage plane, in eggs with bilateral cleavage, appears to mark accurately in almost every case the direction of the median axis of the embryo. This is notably the case in the cleavage of the ctenophores (Metschnikoff, Agassiz, Chun). In the cephalopods bilateral cleavage is again beautifully illustrated (Kölliker, Bobretzky, Vialleton, Ussow, '81; Watasé, '91); though subject to some

individual variation (Watasé, '91), the early cleavage is quite definite, but soon the divisions become too irregular to follow in detail. In the tunicates we find bilateral cleavage of a most conspicuous and determinate type, the median axis of the embryo, as in the preceding forms, being marked out by the first cleavage furrow (Seeliger, van Beneden and Julin, Castle, '96). Among the vertebrates there are no cases of that regularity and determinateness of cleavage which characterize the early development of many invertebrates. The cleavage is quite regular in some forms until a certain period, after which the divisions follow no apparent order. In most forms whose cleavage has been carefully studied there has been found considerable variation in the cleavage of different eggs of the same species. To the extent, however, that the cleavage of the eggs of vertebrates follows any definite plan, it may be said to belong to the bilateral type. The first cleavage plane often coincides with the median axis of the embryo, though in some forms it may apparently form any angle with this axis. In *Amphioxus*, although the form of cleavage is exceedingly variable, exhibiting cases of radial, spiral, and bilateral divisions in different eggs, there is a predominant tendency to bilaterality in the early cleavage (Wilson, '93), and the first cleavage marks the future longitudinal axis. The cleavage of fishes, after the first few divisions, usually becomes quite irregular. In *Batrachus*, according to Miss Clapp, the first cleavage plane may form a considerable angle with the median axis of the embryo. The early cleavage of this form is, nevertheless, quite markedly bilateral, though, as Miss Clapp informs me, it is subject to considerable individual variation, and the plan of cleavage becomes very irregular in later stages. Morgan found, also, in *Fundulus* that, while the cleavage up to a certain stage was of the bilateral type, the first cleavage bears no constant relation to embryonic axes. While in the frog and some other *Amphibia* the first cleavage plane and the median axis of the embryo commonly coincide, the first cleavage plane in *Diemyctylus* (Jordan) and *Triton* (Hertwig) is usually transverse to this axis. The axial relations of the first cleavage in case of the frog, however, are known to be largely influenced by gravity

(Pflüger), and in *Diemyctylus*, Jordan found that the first cleavage plane often deviated considerably ($45-50^\circ$) from the transverse axis.

In *Amblystoma*, Jordan and Eyclescheimer found that the first furrow became so irregular that they considered it improbable that it should ever come to separate, exactly, the right and left halves of the embryo; and the same conclusion may well be drawn from the cleavage of many other vertebrates. Where, in eggs with bilateral cleavage, the first cleavage plane forms an oblique angle with the median axis, this angle varies in different eggs, *i.e.*, the first cleavage plane does not stand at a *constant* oblique angle to the median axis. When the first cleavage plane has *constant* axial relations, it is either median or, more rarely, transverse (*Polychaerus*, Gardiner, '95). Determinate bilateral cleavage may readily be conceived to occur in which the first cleavage furrow stands at a certain constant oblique angle to the median axis, the second at right angles to the first, and only the third or fourth meridional cleavage furrow coinciding with the median plane of the embryo. But the course of bilateral cleavage does not run in this manner. Where, as in the toadfish, the first cleavage plane may form a considerable angle with the median axis, and the cleavages, up to a certain period, are symmetrical in relation to this plane, it is obvious that, unless an extensive shifting of the blastomeres occurs, the cleavage cannot be of the determinate type; cells of corresponding lineage cannot have the same fate in different eggs. In cases like this the form of cleavage can hardly be held to express a bilateral organization of the egg substance and cannot have much morphological significance. Even in eggs devoid of bilateral organization the cleavage might form, according to Sach's law, a more or less definite pattern, whose form would be largely dependent on the amount of yolk in the egg, extrinsic conditions, etc.; or the organization of the egg may be such as to exert no influence on the early cleavage. But in cases where bilateral cleavage is *determinate*, where cells of the same lineage always have the same fate in different eggs, the first cleavage plane apparently always coincides with either the median or the transverse axis of the embryo. Where the first

cleavage plane does not coincide with either of these axes, bilateral cleavage, when it occurs, is of the indeterminate type. The converse proposition also appears to hold good, *viz.*, where the cleavage is determinate and the first cleavage plane coincides with the median or transverse axis, the cleavage is of the bilateral type. If these rules have exceptions, they are sufficiently general not to be devoid of significance. The cleavage of the ovum is influenced by a large number of factors, both internal and external. Of these factors the degree of organization of the egg and the direction of the first cleavage plane with reference to the planes of symmetry of this organization play, I believe, an important part.

Reversal of Cleavage and Cell Homologies.

Detailed study of the early developmental stages of annelids and mollusks has brought to light numerous and striking points of resemblance between the cleavage of various members of these groups; and the cleavage of the polyclades, as shown by Lang's work in *Discocelis*, is so similar to that of the above forms that it may properly be considered as belonging to the same general type. Professor Wilson, in his paper on the "Cell Lineage of *Nereis*," has called attention to the close resemblances of the cleavage of the annelid *Nereis* to that of the gasteropods *Crepidula* and *Neritina* and the polyclade *Discocelis* as follows: "Up to a late stage in the spiral period (twenty-eight cells) every individual blastomere and every cell division is represented by a corresponding blastomere and a corresponding cell division in the embryo of the polyclade, and in that of the gasteropod. In all three the first two cleavages and the upper and lower cross furrows have the same relations. In all, three groups of four micromeres each are successively separated from the macromeres,—the first group in a right-handed spiral, the second in a left-handed spiral, and the third in a right-handed spiral, like the first. The micromeres of the second and third groups alternate with one another so as to form an outer belt of eight cells that surrounds the four primary micromeres."

Professor Wilson also pointed out that in all three groups the first cleavage of the first and third quartettes occurs in the same direction, and that the cleavage of the gasteropods and the annelid is characterized by an additional point of agreement, in that the primary mesoblast cell, *4d*, is given off in both cases in a left-handed spiral, from the left posterior macromere *D*.

It was found later by Lillie ('95) that the cleavage of *Unio*, one of the lamellibranchs, agrees with that of the gasteropods and annelids, not only in the features mentioned, but in several other points of undoubted morphological significance; and Conklin has since added many other striking resemblances which characterize the cleavage of annelids and gasteropods. The discovery made by Conklin that the turret cells in *Crepidula* have the same origin, position, and, in the main, the same fate as the trochoblasts of the annelids, and that the prototroch, in both groups, is completed by certain cells of the second quartette, affords one of the most notable points of similarity in the cleavage of these forms.

Resemblances such as these would seem to indicate, as Conklin strenuously insists, that cleavage has a much greater morphological significance than has usually been assigned to it. Yet, while recognizing all these wonderful similarities between the cleavage of different classes of animals, does it follow that the form of cleavage has any really fundamental connection with the process of development? While it is true that there are numerous cases in which cells of corresponding origin and position have the same fate in widely separated groups, there are other instances in which cells of the same origin have a very different fate in forms which are much more closely allied. (Compare the fate of the larval mesoblast cell in *Unio* with that of the same cell in *Planorbis*.) And there are also cases in which it has been found that cells which have the same fate have a quite different origin, even in the same class of animals; instance the origin of the secondary mesoblast in *Crepidula* and *Planorbis*. It is hard to reconcile these facts on the view that the process of cleavage has any fundamental connection with the homology of organs. Neither can the similarity in the cleavage of different groups be accounted for by attributing it

to extrinsic mechanical conditions. The resemblances between the cleavage of mollusks and annelids are too numerous and too close to be explained in this manner, or even, as I believe, by the principle of "parallel precocious segregation." But while similarity of cleavage in different groups, provided it is long continued and close, may be held to indicate genetic affinity, the converse of the proposition, that differences in the form of cleavage imply lack of relationship, does not always follow. It is well known that the form of cleavage may vary even in eggs of the same species. The summer and winter eggs of daphnids have entirely different forms of cleavage, yet both develop into the same kind of embryos. In many vertebrates the particular manner in which the egg is divided appears to be a matter of little moment as regards its future development. In Renilla, Wilson found that the early cleavage of the egg presented great variations which were without any apparent influence on the end result.

It is obvious that no general rule can be drawn regarding the phyletic significance of cleavage. In some groups cleavage has, doubtless, a high degree of "systematic worth"; in others it may have very little. Similarly, as a mark of affinity between the different groups of animals, as in the case of gasteropods and annelids, cleavage may be a character of considerable value; or again, as in the case of the gasteropods and cephalopods, its evidence may be of little weight. It seems probable that similarities of cleavage should be regarded as an *incidental* and not a *necessary* expression of genetic affinity. Whether or not the relationship between different classes of animals expresses itself in the early cleavage of the ovum may depend largely upon external conditions, or upon the amount of yolk in the egg, or, perhaps, upon the degree of cytoplasmic differentiation that has been reached before cleavage begins. It is not my purpose, however, to attempt to discuss what may be the reason for the varying morphological significance of cleavage forms in different groups of animals. The fact I would emphasize is that mere cell genealogy stands in no *necessary* relation to the genealogy of organs. This conclusion, which is supported by a variety of considerations, receives a strong confirmation — if

not a demonstration — through the facts of reversal of cleavage. It can readily be seen that, by virtue of reversed cleavage, the relative positions of certain cells become different from those they would occupy if the cleavage were of the normal or unreversed type. For example, the position of the cells of the third quartette in each quadrant is, in the dextral forms, to the right, and, in the reversed forms, to the left of the cells of the second quartette. Similarly, the trochoblasts lie to the right of the apical cells in the dextral forms, and to the left of these cells in the sinistral forms. In the dextral forms the cells in the two anterior quadrants of the third quartette are $3a$ and $3b$, and in the posterior quadrants $3c$ and $3d$. In the reversed forms $3b$ and $3c$ are anterior, and $3a$ and $3d$ posterior. In the dextral forms the trochoblasts are $1a^2$, $1b^2$, anterior, and $1c^2$, $1d^2$, posterior; while in the reversed forms the anterior trochoblasts are $1b^2$, $1c^2$, the posterior, $1a^2$, $1d^2$. In both reversed and unreversed forms the corresponding arms of the cross are derived from the same quadrants.

The anterior trochoblasts in both *Crepidula* and *Planorbis* go to form the prototroch, and the posterior ones go into the head vesicle. The cells which have a similar position in the two forms have the same fate, although they have a different origin. The cells which go into the prototroch in *Crepidula* are $1a^2$ and $1b^2$, while in *Planorbis* the cells which have this fate are $1b^2$ and $1c^2$. Conversely, cells of the same cell origin have different fates, *viz.*, $1a^2$ goes into the prototroch in *Crepidula*, while in *Planorbis* it forms a part of the head vesicle. Although the cell $1b^2$ goes into the prototroch in both forms, it forms a part of the *right* side of this structure in *Crepidula* and a part of the *left* side in *Planorbis*. It certainly appears that, in a certain sense, the fate of a cell is a function of its position. It has been remarked by Driesch that, if the blastomeres of an egg could be shifted about at will, their development would take place in accordance with their relative positions. While in reversed cleavage nature has performed an experiment for us in the shifting of blastomeres, and while the results show that the fate of the cells is in accordance with their position, and not their genealogy, the experiment differs considerably from

the hypothetical shifting process of Driesch. The blastomeres are not shifted about promiscuously, but cells occupying similar positions to those in the unreversed eggs contain cytoplasm derived approximately from the same portion of the ovum; and it may be for this reason, and not on account of the mere fact of position, that they come to have the same destiny. Corresponding portions of the egg cytoplasm develop along similar lines, whose direction appears to be independent of the precise form of cleavage, even when the cleavage is of a highly determinate type. The direction of every cell division may be reversed up to a late period of cleavage without altering the fate of the cells having the same position in the egg.

Reversal of cleavage, like the pressure experiments of Driesch on echinoderm eggs, and of Wilson on the eggs of *Nereis*, shows that the immediate causes of the differentiation of cells lie, not in the nucleus, but in the cytoplasm. While certain cells have the same position and fate in reversed and unreversed eggs, the nuclei of these cells have entirely different lines of descent; and, conversely, nuclei having the same origin come to lie in entirely different portions of the embryo. Thus, in perfectly normal development, the fate of cells appears to be entirely independent of the origin of their nuclei. How this fact can be reconciled with the view that the differentiation of blastomeres is mainly the result of qualitative nuclear divisions, I cannot understand, unless we suppose that there is some complex mechanism for the proper sorting of nuclear material to provide for the contingency of reversed cleavage.

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EXPLANATION OF PLATE XVII.

- FIG. 1. Egg mass of *Planorbis trivolvis*.
FIG. 2. Undivided egg.
FIG. 3. 2-cell stage, showing the second cleavage spindles and the torsion of the dividing cells.
FIG. 4. 4-cell stage viewed from the side, showing the laeotropic division of *B*.
FIG. 5. The same egg seen from the upper pole, showing the laeotropic inclination of the spindles.
FIG. 6. 8-cell stage seen from the side.
FIG. 7. The same egg seen from the animal pole.
FIG. 8. Apical view of the 24-cell stage.
FIG. 9. Same egg seen from the side.
FIG. 10. Same egg seen from the vegetal pole, showing the division of *D* which produces the primary mesoblast cell *D*⁴ or *M*.
FIG. 11. View of the lower pole of the 33-cell stage, showing the large mesoblast cell lying partly in the cleavage cavity.
FIG. 12. Lateral view of the same egg, showing the cleavage of the lower tier of the second quartette.

EXPLANATION OF PLATE XVIII.

FIG. 13. Apical view of an egg in the 49-cell stage.

FIG. 14. Lower side of same egg, showing the mesoblastic pole cells, M^1 and M^2 , lying partly in the cleavage cavity.

FIGS. 15, 16, and 17. Lateral views of the same egg.

FIG. 18. Egg of about 54 cells seen from the vegetal pole. The cells of the fourth quartette are seen in process of division.

FIG. 19. Lateral view of same egg, showing dextrotropic cleavage of $2a^{1.2.1}$. By an error the same figure is repeated in Fig. 20.

FIG. 21. Apical view of an egg of about 64 cells, showing a division of two of the basal cells of the cross.

FIG. 22. View of posterior side of the same egg. The portion of the mesoblastic pole cells, M^1 and M^2 , exposed at the surface of the egg, has become considerably reduced.

FIG. 23. Lateral view of an egg of about 80 cells, showing the division of the upper pair of cells of the third quartette in the α quadrant.

FIG. 24. Vegetal pole of an egg of about the same stage. The mesoblastic pole cells have entirely disappeared from the surface.

EXPLANATION OF PLATE XIX.

FIG. 25. Apical view of an egg of 104 cells. The trochoblasts have increased in size, become clearer, and have apparently compressed the arms of the cross which stand out in greater contrast to the cells between them than in the earlier stages of development.

FIGS. 26, 27, 28, and 29. Lateral views of the same egg. It will be seen that the cells of the second quartette in the *b* quadrant have not kept pace with the divisions of those of the other three quadrants. In Fig. 29, $3a^{1.2}$ has divided, while its fellow, $3a^{1.1}$, remains entire; the corresponding divisions have both occurred in the *d* quadrant.

FIG. 30. Lower pole of same egg. The cells of the fourth quartette have undergone a second cleavage.

FIG. 31. Apical pole of an egg of 130 cells. The tip cells of the cross have enlarged and become clear, the arms show a marked laeotropic twist, and the splitting of the arms is begun by a transverse division of one of the cells of the anterior arm. The apical pole has begun to rotate forward, and the posterior trochoblasts have become slightly larger than the others.

FIG. 32. Left side of same egg.

FIG. 33. Anterior side of same egg. The third quartette in the *b* quadrant consists of a double row of four cells. In the *c* quadrant the divisions of the third quartette are somewhat more advanced. $3c^{2.2.1}$ has just divided, while the corresponding cell, $3c^{2.1.1}$, is entire. $3c^{1.2.2}$ has divided, and its fellow, $3c^{1.1.2}$, is undergoing division. The upper cells of the second quartette are beginning to become clear and to take on the character of the neighboring trochoblasts.

FIG. 34. Right side of same egg; the forward rotation of the apical pole may be seen here as in Fig. 32.

FIG. 35. Posterior side of same egg.

FIG. 36. Vegetal pole of same egg. A fifth quartette has been formed, and the cells of the fourth quartette in the *b* quadrant have undergone a division at right angles to the previous one; the corresponding cells in the *a* and *c* quadrants remain undivided.



EXPLANATION OF PLATE XX.

FIG. 37. Apical view of an egg of about 150 cells. $2b^{1.1}$ is in process of division, although this change had taken place in the earlier stage shown in Fig. 31. A longitudinal splitting has taken place at the base of the lateral arms of the cross.

FIG. 38. Vegetal pole of the same egg. The lower side is flattened and slightly depressed in the center. In the b quadrant two of the secondary mesoblast cells have lost connection with the surface, and two others, $3b^{2.2.1.2}$ and $3b^{2.1.1.2}$, have still a small portion of their surface visible at the outside of the egg.

FIG. 39. Anterior view of an egg of about the same stage as the preceding. The tip cells of the anterior arm of the cross have become clear, and are being pushed by each other by the forward rotation of the apical pole. The lower cells of the second quartette are somewhat dislocated by the same process.

FIG. 40. Apical view of a later stage, showing the two upper cells of the second quartette of the anterior side of the egg now lying side by side.

FIG. 41. Vegetal pole of an egg of about the same stage. The depression of the endoterm is deeper than in Fig. 38. A division has apparently occurred in $2b^{2.2.1}$ and in $1b^{2.2.1}$. The middle cells of the anterior quadrant of the second quartette, $2b^{2.1.1}$, $2b^{2.1.2}$, $2b^{1.2.1}$, and $2b^{1.2.2}$, have been dislocated by the rotation of the apical pole so that they lie in a transverse line. The secondary mesoblast cells no longer appear at the surface of the egg. The stomatoblasts in the a , b , and c quadrants still remain undivided, while the corresponding cell of the d quadrant is crowded away from the entomeres by the cells of the third quartette, which come to meet in the middle line (*cf.* Figs. 30, 36, 38, and 41).

FIG. 42. Apical pole of a later stage. The anterior rotation of the apical pole is increased, and the cells of the middle of the cross are beginning to enlarge and become clear.

FIG. 43. Right side of same egg.

FIG. 44. Lower pole of same egg; mouth of gastrula reduced to a slit.

FIG. 45. Posterior side of a gastrula, showing the large head vesicle.

FIG. 46. Ventral side of the same gastrula, showing the blastopore reduced to a minute slit lying between a pair of oblong cells. Prototroch shown by a band of clear cells in front of the blastopore. Rudiments of the cerebral ganglia shown by two patches of dark cells separated by a median band of large, clear cells, the apical plate *A.P.*

FIG. 47. A later stage, showing the prototroch, apical plate, *A.P.*, blastopore, *M.*, and the rudiment of the right cerebral ganglion. The cell boundaries shown in the figures in this plate are not diagrammatic, but represent accurately the outlines of every cell. In eggs stained with silver nitrate these boundaries are as clear as shown in the figures.

FIG. 48. View of anterior portion of a gastrula, showing the cell $1b^{1.2.1.1}$ pushed forward until it comes in contact with the prototroch.

59.

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7.

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EXPLANATION OF PLATE XXI.

FIG. 49. Mesoblastic pole cells, showing the small cells budded off at the anterior end.

FIG. 50. Optical section of an egg of about the stage shown in Fig. 41. *P.M.*, primary mesoblast; *S.M.*, secondary mesoblast.

FIG. 51. Posterior side of a young embryo, showing the large head vesicle, *h.v.*, and the beginning of the shell gland, *s.g.*

FIG. 52. Optical section of a slightly later stage. *M.*, mouth; *s.g.*, shell gland; *c.g.*, rudiment of cerebral ganglion which is proliferating cells; *p.*, prototroch; *e.c.*, entodermic cells that have become gorged with albuminous matter; *mes.*, cells of the mesoblastic band; *g.c.*, giant cell that has become partially perforated.

FIG. 53. Surface view of the same stage. *M.*, mouth; *p.*, prototroch; *c.g.*, rudiment of cerebral ganglion. The patch of clear cells around the mouth gradually narrows posteriorly until it becomes a narrow band of clear cells separating the two halves of the foot.



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